CHANGES IN 40 CPS EEG ACTIVITY IN THE OLFACTORY BULB FOLLOWING GAMMA IRRADIATION OF THE CAT

A Dissertation

Presented to

the Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Barbara Piper Uzzell

August, 1970

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ABSTRACT

The initial electrical, chemical, and behavioral changes following gamma irradiation were investigated with five total dose groups of 17 mongrel, male cats that received either sham irradiation, 500 r. whole body (WB), 800 r. head only (HO), 1000 r. HO, or 2000-3000 r. HO irradiation. During preliminary investigation, 800 r. and 1000 r. HO irradiated animals were trained to perform 30 min. daily sessions on a FR-5 schedule for milk reinforcement and EEG recordings were taken during performance. For the major investigation, four 500 r. WB, six 2000-3000 r. HO, and two sham irradiated animals were trained to perform a visual discrimination task which required the animals to press a bar for milk reinforcement during the presence of a 10 sec. 7 cps. flashing light. Fifty daily trials were continued until the animals were making less than one intertrial response for every reinforced response as determined by a criterion ratio during three consecutive sessions. Total number of responses, intertrial responses, and reinforced responses were recorded following each daily session.

Following criterion performance, EEG recordings were taken on the unrestrained animals during five performance sessions on the task. The animals were exposed to Co^{60} sources at Texas A. & M. University. The dose rates for these exposures were 60 and 90 r./min. Total doses were

determined by exposure time. Subsequently 3, 24, 48 hour and 7-8 day EEG recordings were taken while the animals were performing the behavioral task. Six chronically implanted and three nonelectrode animals receiving 2000-3000 r. HO or sham irradiation were sacrificed at 3, 24 hours and 8 days for serotonin and norepinephrine assays of olfactory tissue.

The EEG recordings and computerized frequency analysis of the 2000-3000 r. HO animals showed a loss of the 40 cps. activity in the olfactory bulb 3 and 24 hours after irradiation during 10 sec. nonstimulation and stimulation periods of a trial. The loss of the 40 cps. activity appeared in the EEG recordings and computerized frequency analysis of the WB animals 7 days after irradiation during 10 sec. nonstimulation and stimulation periods of a trial. No 40 cps. changes were observed up to 7 days after 800 and 1000 r. HO irradiation. Serotonin content of the olfactory tissue tended to increase and norepinephrine content tended to decrease slightly at the 3 hour period following irradiation. No impairments in behavioral performance were detected until radiation sickness appeared.

The 40 cps. loss was attributed to increased serotonin content in the olfactory structures. Behavior as measured by performance on a visual discrimination task was unrelated to the initial electrical and chemical changes.

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CHAPTER I

STATEMENT OF THE PROBLEM

Memory with its complex interrelated functions of registration, consolidation, storage, and retrieval has evaded elucidation of precise research and perplexed the most ardent researcher. Determining the process and mechanisms involved in memory has been a very obvious intent of most research concerned with brain and behavioral relations.

Various approaches have been applied to the problem of memory processes. One approach has been to correlate the various characteristics of brain electricity with behavior. The approach has shown that 40 cps. synchronous EEG activity in the olfactory bulbs, the prepyriform cortex, basolateral amygdala, and a lesser extent in the corticomedial amygdala was related to nasal airflow (Sheer, Benignus, & Grandstaff, 1963); the relationship between 40 cps. activity and these structures; the conditions for the occurrence of 40 cps. activity in the amygdala (Grandstaff, 1965); the relationship between 40 cps. rhythm in the amygdala and a primary input station of a conditioned stimulus (Sheer, Grandstaff, & Benignus, 1966); and the relationship between 40 cps. activity in the primary input station and the response area after learning had occurred (Sheer & Grandstaff, 1970). Frequencies centered at 40 cps. may be considered an index of the ongoing consolidation of short term memory (Sheer, 1970).

The same approach is taken in the present study, but in addition to the relationships between electrical brain activity and behavior, some chemical constituents of the brain were considered. More specifically, the study was concerned with the relationships between 40 cps. activity in the olfactory bulbs, behavioral performance on an appetitive task, and brain chemistry. These relationships were assessed with and without gamma irradiation in an attempt to describe some of the effects of ionizing radiation on the central nervous system (CNS) and to contribute some information to the elusive concept of memory consolidation.

To actuate the design, a group of cats were implanted with multiple electrodes for recording during the performance of a visual discrimination task. The EEG recordings were placed on magnetic tape and subjected to spectrum analysis. The animals were irradiated by a Co^{60} source at Texas A. & M. University. Concentrations of serotonin and norepinephrine were determined for some olfactory structures.

CHAPTER II

REVIEW OF THE LITERATURE

Research in the area of radiobiology has been aimed at delineating changes in a biological system that were solely attributable to exposure to some radioactive source. Certainly enough exposure would be debilitating and may result in death to the organism. Research describing the changes which are incurred from exposure and the mechanisms responsible for the changes is still in progress, although much knowledge has been gained from past endeavors.

Radiation changes are capricious. The changes may be evident immediately following exposure or after some latent period. Besides the time factor, research endeavors have added to the complexity by varying exposure time, total dose, dose rate, and selecting certain regions of the organism for exposure. This is not to say that variance of radiation variables is distasteful, but rather it sometimes thwarts a complete glimpse of the changes associated with exposure to a radioactive source. Certainly from a practical point of view, in the treatment of cancer, research variance of radiation variables may be most helpful to the radiologist who is faced with a formidable problem in the treatment of a patient (Lascelles, 1968; Jelliffe, 1968).

The present review attempts to assist the reader in obtaining a more complete view of the changes attributed to

exposure of an ionizing source by considering the electrical, chemical, and behavioral literature. The structural relationships of the olfactory bulb were also considered since the olfactory area has been of interest to researchers in radiobiology and the electrical data presented in the present study were obtained from the olfactory bulbs.

The research from the Soviet Union was purposely overlooked in the following discussion. The rationale for the neglect involved the following reasons: 1) Statistical analyses of the data were omitted from the reports; "typical" results were presented and variability was sometimes ignored. 2) Instrumentation, procedures, dosimetry were ignored. 3) Effects of lower dose levels may not be applicable to those of the present study. 4) The general health of the experimental animals after irradiation was ignored (Furchtgott, 1963).

STRUCTURAL RELATIONS OF THE OLFACTORY BULB

An important part of the behaviors of most animals has been associated with the sense of smell, particularly feeding and courtship behaviors. The dominance of the olfactory system has been noted in animals by the size of the olfactory system in relation to the rest of the brain. Mammals have been classified with such terms as macrosmatic, microsmatic, and anosmatic according to the relative size of the olfactory structures. In spite of its importance, the olfactory sense

has become probably the least understood of all special senses. Properties which give substances their characteristic odors have not been delineated (Beidler, 1961; De Vries & Stuiver, 1961).

The olfactory system has an unchanging nature in a wide range of animals. Examination of the peripheral system in mammals reveals a large nasal cavity with folds of turbinal bones lining its walls. The olfactory epithelium is located in the posterior portion of the cavity on the ethmoturbinal bone as well as along the posterior half of the nasoturbinal (which extends along the roof of the nasal cavity in both chambers). The olfactory epithelium which is distributed over the pigmented mucous membrane consists of three types of cells: receptor, supporting and basal. In macrosmatic animals the pigmentation is brown or dark yellow.

The supporting cells have cylindrical shape and the basal portion appears to be forked when compressed between the bodies of the receptor cells. The cytoplasm of these cells has fine granules of yellow and brown pigmentation. A row of small cells on the basement membrane have been named the basal cells. The receptor cells have been described as bipolar neurons with a spindle shaped body. A slight enlargement at the external limiting membrane of these cells contain 8 to 12 fine hairs. These receptor cells are quite unique since they serve both as receptors and conductors without a single synapse to the olfactory bulb.

Bowman's glands which lie on the olfactory epithelium disappear where the respiratory mucosa begins. The function of these glands is to bathe the whole olfactory epithelium. For stimulation of the receptor cells, odor particles must dissolve in the secretion (Allison, 1953).

Animals, such as the cat, have an accessory olfactory organ which has the shape of a long, thin tube lined by epithelium. The accessory organ has a protective bone and cartilage covering. The function of the vomeronasal organ has not been determined and it appears to be heavily vasculated.

The axons of the receptor cells form the olfactory nerve fasciculi which passes through foramina in the ethmoid bone to the outermost layer of the bulb. Some topographical projections from the epithelium to the olfactory bulb have been demonstrated (Adrian, 1950) but not as precisely as the projections in the visual system.

The olfactory bulb is a rather prominent structure in a macrosmatic animal such as the cat. Dorsally the bulb is attached to the frontal pole of the cortex and ventrally to the olfactory crus. The fissura circularis separates the bulb and the olfactory crus. The dorsal surface of the bulb is in a more anterior plane than its ventral caudal pole.

On the dorsomedial surface as the bulb narrows posteriorly, lays a small structure which receives the entering vomeronasal nerve. The small structure is known as the

accessory bulb which differs in details of internal structure from the main bulb, although the general pattern is the same (Allison, 1953).

The structure of the olfactory bulb consists of distinct layers of lamination. The number of these layers varies from anatomist to anatomist. Fox (1940) has chosen seven layers from his studies of the olfactory bulb of the cat. From the periphery, these laminated layers include: 1) entering unmyelinated nerve fibers; 2) glomeruli layer; 3) external plexiform layer; 4) mitral cell layer; 5) plexiform layer; 6) granule cell layer; and 7) ependymal layer.

Nerve fibers entering the first layer proceed from a short number of main branches which divide at some distance from the glomerulus into brush endings. Each glomerulus is formed by several brush endings. Dendritic branches of the mitral cells are found in this layer. The relationship between the dendritic branches and the glomerulus may not always be one-to-one. Rather the glomerulus may receive two dendritic branches of a single mitral cell or three different glomeruli may possess the dendritic branches from a single mitral cell (Valverde, 1965). Another structural component of the glomeruli layer is the short axoned periglomerular cells or external granule cells which connect the glomeruli in this layer.

The third layer or the external plexiform layer consists of randomly disposed tufted cells and dendritic branches of

the mitral cells. The tufted cells have fusiform bodies with dendrities coursing toward the glomerular layer. The axons of the tufted cells are smaller than those of the mitral cells. Some of the axons penetrate among the glomeruli, while others ascend to the internal plexiform layer. The axons of the tufted cells do not seem to leave the olfactory bulb and the cells appear to be inwardly displaced peri-glomerular cells, not outwardly displaced mitral cells (Valverde, 1965).

The mitral cells in the fourth layer appear as a row of large triangular bodies with apexes pointing inward. The axons of the mitral cells are large and thick (Valverde, 1965), not thin and difficult to follow as earlier believed (Allison, 1953). The axons enter into the internal plexiform layer and provide collaterals here and there to form part of the dense fibrillar network of this layer. The axons of the tufted cells and extrinsic fibers from the outside of the olfactory bulb form the dense plexus of the fifth layer in addition to the axons and collaterals of the mitral cells.

The distinction between the internal plexiform layer and the granule cells is not always precise. The granule cells have medium size egg-shaped bodies which lack expansions of axons. The descending expansion of a granule cell functions as a true axon by transmitting the activity of the internal plexiform layer to the dendrities of the mitral cell, although morphologically the expansion is not an axon. The

descending expansion reaches the layer of the mitral cells where it divides into several secondary branches. Some of these descending expansions of the granule cells appear to have axo-axonic synapses with the mitral cells as well as axo-axonic synapses with the afferent fibers from the anterior olfactory nucleus. The latter axons may provide central modulation of the sensory inflow (Valverde, 1965; Powell, Cowan, & Raisman, 1965; Price, 1968). The axo-axonal synapses suggested presynaptic inhibition (Eccles, 1964) may exist in the bulb (Phillips, Powell, & Shepherd, 1961; Yamamoto, Yamamoto, & Iwana, 1963; Shepherd, 1963, 1966; Baumgarten, Green, & Mancia, 1962).

The dendritics of the granule cells have a brush appearance covered by numerous fine sprouts which point toward the ventricular cleft of the olfactory bulb. Medullated fibers which enter the olfactory tract appear to begin in the inner side of the granule cells.

The lateral, medial, and intermediate olfactory tracts have been recognized in the cat. The lateral olfactory tract arises from the dorsal and ventral peduncles. The peduncles have their beginnings with the fibers from the medial and lateral sides of the ventricle of the olfactory bulb. The ventral peduncle contributes fibers to the ventral section of the lateral olfactory tract--first the fibers from the lateral side of the bulb, then the ventral side of the bulb, and finally the medial side of the bulb. The dorsal peduncle

carries fibers from the accessory bulb and contributes to the dorsal section of the lateral olfactory tract. In the olfactory crus, the pars lateralis contributes fibers to the lateral olfactory tract. The lateral olfactory fibers continue caudally between the olfactory tubercle and the pyriform lobe and distributes to certain amygdaloid nuclei.

The intermediate olfactory tract arises dorsal, dorsolateral, and finally lateral to the olfactory ventricle. It contains fibers to the pars posterior of the anterior olfactory nucleus in the olfactory crus, as well as fibers from the pars dorsalis and pars lateralis of the anterior olfactory nucleus. The intermediate olfactory tract passes to the anterior portion of the olfactory tubercle and the pyriform cortex. The latter two contribute fibers to the tract. The tract turns dorsomedially under the head of the caudate nucleus to enter the anterior commissure and become the most rostral component of that structure.

The medial olfactory tract, which is small in comparison to the other two tracts, lies in the ventromedial portion of the crus. It soon disappears after having connections with the anterior continuation of the hippocampus.

The anterior olfactory nucleus lies in the tissue connecting the bulb with the ventral part of the hemisphere. It contains several subgroups of cells which gradually appear in transverse sections of the olfactory bulb until they form a ring of grey around the olfactory ventricle. These subgroups

include the pars lateralis, pars dorsalis, pars medialis, and pars ventralis. Each of these subgroups fuse with other structures--the pars lateralis with the pyriform cortex; the pars dorsalis with the frontal cortex; the pars medialis with the anterior continuation of the hippocampus; and the pars ventralis with the olfactory tubercle. The pars lateralis and the pars dorsalis are proportionately large in the cat, while the pars medialis is small (Fox, 1940).

Two further subgroups of the anterior olfactory nucleus have been determined. These include the pars externa which lies in the path of the ventral and dorsal peduncles of the olfactory tract, and the pars posterior which occupies a position near the head of the caudate nucleus.

Valverde (1965) suggested the existence of an inter-bulbar path in the rat via the anterior olfactory nucleus and the anterior commissure. The existence of such a pathway in the cat has been shown (Fox & Schmitz, 1943).

ELECTROPHYSIOLOGICAL RADIATION STUDIES

Typically radiation research has been concerned with demonstrating EEG changes in a specified time period following irradiation. Acceleration of the usual procedure by continuous exposure of guinea pigs to X-rays until death produced an EEG shift towards slower waves after a total dose of 2.5 kR. (Andrews & Brace, 1953). The report of a preponderance of slow waves was to occur again and again in later EEG studies.

Early EEG radiation researchers were concerned with demonstrating cortical changes following irradiation of an animal. At first, an immediate report of EEG changes seem impossible. Following 700 and 800 whole body (WB) doses, the EEG changes appeared only when the monkeys reached preterminal stages of illness (Eldred & Trowbridge, 1953). Using larger doses and a Ba^{140}-La^{140} source, Brooks (1956) found frequency and voltage depressions in the frontal, parietal, and occipital regions within one minute after WB irradiation of monkeys. Recovery occurred within one minute and remained for the 100 minute recording period after doses of 2.5 kR. or less. Transient recovery occurred in the presence of continued radiation after 5.0 kR. and was followed by a second depression which remained throughout the recording period. A second major EEG change had appeared. Not only did voltage and frequency changes occur in the EEG recordings after irradiation, but these changes were transitory.

While EEG radiation research was still concerned with cortical changes, an attempt was made to localize exposure and correlate the changes with the total dose an animal received. Head only (HO) irradiation was chosen for the correlation task and the doses ranged between 1.5 and 6.0 kR. The major EEG changes for the 6.0 to 4.5 kR. group occurred within the first 24 hours. These changes included periodic spiking and generalized slowing of activity together with an increase in amplitude. After 48 hours, 3-5 cps. activity

predominated with increased amplitude. Following exposure to 3.0 kR., no EEG changes were observed within the first six hours; after 24 hours medium to high amplitude 3-5 cps. activity appeared. Following exposures of 2.0 and 1.5 kR., the high amplitude 3-5 cps. activity appeared in the EEG recording on the fourth day (Ross, Leavitt, Holst, & Clemente, 1954). Amplitude changes have been observed following unilateral exposures of 3.5 kR. to the cortex of monkeys. These localized changes were more readily recorded during drowsiness and sleep than during the waking state. Sleep spindles were either reduced or absent in the irradiated area (Caveness, Roizin, Carsten, & Schade, 1964).

Transient cortical changes have been observed in rats, as well as monkeys, following WB exposures to 700 R. The analysis of the EEG activity was not described only in terms of frequency and voltage changes, but grouped into two frequency bands, a high (14-30 cps.) and a low (1.5-7.0 cps.). Both of these bands decreased within three hours of irradiation and returned to normal levels during the next 1 to 2 days. During the third day the low frequency activity was depressed and remained low for the duration of the experiment. Terminal morbidity 10-11 days later was associated with an increase in high frequency activity (Caster, Redgate, & Armstrong, 1958).

EEG radiation research became concerned with more than demonstrating cortical changes and electrodes were implanted

in subcortical structures. Along with subcortical recordings came electric stimulators to determine the effects of ionizing radiation on rabbits, cats, and dogs. Spontaneous activity from cortical and subcortical areas was immediately characterized by low voltage, fast activity after a 400 r. WB dose of cats and 400 or 600 r. HO dose of rabbits. Total WB doses of 200 r. produced no changes in the EEG recordings of cats while 900 r. HO doses of rabbits produced high voltage, slow activity. Hippocampal spikes were evident, particularly during sleep, and increased for the 400 r. HO, 400 r. WB, and 200 r. WB groups of cats. No spikes occurred in the hippocampal recordings of cats receiving 400 r. body only (BO) doses. Hippocampal spikes were observed following 400, 600, and 900 r. HO irradiation of conscious rabbits and following 10 and 100 r. exposures of sleeping and aroused dogs. The time course for the maximum firing rates was different for each dose group. Brainstem reticular thresholds decreased following WB irradiation of cats, but not after HO exposures. Midbrain reticular thresholds decreased following 400 and 600 r. HO irradiation and increased after 900 r. HO irradiation of rabbits (Gangloff & Haley, 1960; Monnier & Krupp, 1961; Sams, Endo, Aird, Adams, & Ellman, 1966). The sensitivity of the CNS to electroshock following irradiation has been demonstrated (Rosenthal & Timiras, 1961).

Lower doses of irradiation have tended to produce a desynchronized EEG pattern and arousal in sleeping rats and

suggested peripheral sensory systems had been stimulated. In most of the earlier cortical changes had been noticed in the occipital recordings. This was not too surprising since radiation effects at the receptor level had been demonstrated (Brown, Cibis, & Pickering, 1955; Lipetz, 1955; Veniga, 1961; Bachofer, 1962; Devi, Riley, & Burns, 1968). More recently a computerized statistical technique has demonstrated that the linearly shared activity between the lateral geniculate and the visual cortex decreased following 400 R. HO irradiation of cats (Christensen, Smith, & Haley, 1969). But the desynchronized EEG pattern persisted following 10 second exposures of 0.5, 0.2, and 0.1 r./sec. in blind rats (Garcia, Buchwald, Bach-y-Rita, Feder, & Koelling, 1963).

To verify the responsiveness of peripheral sensory systems to ionizing radiation, spinal animals received low doses of X-irradiation. WB and HO exposures of the spinal transected animals produced the usual desynchronized pattern. No EEG changes occurred in spinal animals that received BO exposures but irradiation of animals with the spinal cord intact produced the change (Cooper & Kimeldorf, 1964). Substantial evidence for immediate radiation intervention in neural or direct sensory systems was accumulating electrophysiologically (Garcia & Buchwald, 1963; Kaack, 1967), behaviorally (Garcia, Kimeldorf, & Koelling, 1955; Garcia, Kimeldorf, & Hunt, 1961; Garcia, Buchwald, Feder, & Koelling, 1962; Harlow, 1962; Hunt & Kimeldorf, 1967), and autonomically

(Hunt & Kimeldorf, 1962, 1963; Inada & Yoshimi, 1965).

In one behavioral experiment in which a narrow, collimated X-ray beam was used as the conditioned stimulus (CS) and electric shock as the unconditioned stimulus (UCS), the conditioned suppression of a bar press response was obtained most often when the X-ray beam passed through the olfactory bulb region (Garcia, Buchwald, Feder, Koelling, & Tedrow, 1964). Subsequently ablations of the olfactory bulbs was found to reduce the EEG desynchronized pattern (Cooper & Kimeldorf, 1964; Hull, Garcia, Buchwald, Dubrowsky, & Feder, 1965) and the loss of behavioral detection (Dinc & Smith, 1965; Brust-Carmona, Kasprzak, & Gasteiger, 1966).

The direction of the following research was to determine the olfactory mechanism responsible for the electrical changes following exposure to ionizing radiation. Extracellular microelectrode recordings from single neurons in the mitral layer, granule layer, external plexiform layer, and glomerular layer of the olfactory bulb indicated that most units responded to WB, HO, and olfactory bulb irradiation with frequency increases. After exposure was terminated, the rate was slower than the preirradiated control rate for a period of about five seconds. No unit responses were observed when exposures included BO, a head region posterior to the olfactory bulb, or a region 5 or 8 mm. anterior to the head. Ethyl alcohol perfused through the nasal cavities abolished all unit responses to irradiation and saline temporarily

abolished the response (Cooper & Kimeldorf, 1966). Similar responsiveness of single units in the olfactory bulbs was obtained from rabbits, dogs, and cats with the highest response frequency occurring in the cat (Cooper & Kimeldorf, 1967). The firing rate of an olfactory bulb unit during irradiation was found to be a linear function of the logarithm of the dose rate, and the threshold response was a constant product of the dose rate and exposure duration (Cooper, 1968). Both these findings are well known functions in sensory physiology--the Weber-Fechner function and the Bunsen-Roscoe relation. Furthermore responses of the olfactory bulb neurons to β-irradiation occurred only when the beam was focused on the olfactory epithelium. Receptor effects have been suggested with another species and a much larger dose (Brunst, 1965). Sensitivity has been shown by the lack of olfactory discrimination in adulthood following prenatal irradiation (Furchtgott & Kimbrell, 1967).

The responsiveness of olfactory receptors suggested stimulation of the structures during irradiation. Ozone inhalation has been suspected of olfactory stimulation during irradiation (Gasteiger & Helling, 1966; Helling & Gasteiger, 1967), and some controls have been provided. Five control rats with chronic tracheotomies which prevented nasal inhalation or any other radiation by products were included in the experiment of Hull et al. (1965). All five animals showed EEG arousal to X-rays.

Air, oxygen, argon, and nitrogen have been perfused through the nasal cavities in order to determine the responsiveness of the olfactory neurons to a particular gas during irradiation. Although many neurons responded to all four gases, the responses were depressed during argon or nitrogen perfusion relative to those during air and oxygen (Cooper, Kimeldorf, & McCorley, 1966). Nasal oxygen concentrations have been implicated in the responsiveness of olfactory bulb neurons during irradiation (Cooper, 1970).

At higher doses of irradiation, the importance of the receptor seems to diminish, thereby suggesting two processes, one activated at low dose levels and another at higher dose levels of irradiation. Large sinusoidal potentials having a frequency of 30-40 cps. from the olfactory bulbs of rabbits were abolished by 50 to 70 kR. HO irradiation. Even higher doses were necessary to abolish all neuronal responses to odor as determined by microelectrode recordings of a single olfactory unit. Response failure seemed to be related to the effects of irradiation of the olfactory bulb since olfactory receptor responses and the large slow, surface potential of the olfactory mucosa were still functional (Cooper, 1969).

A related responsiveness to ionizing radiation has been obtained when the dose rate and the total dose were varied. In the prepyriform area which has connections to the olfactory bulb, the spontaneous activity is also dominated by 40-50 cps. sinusoidal waves. The spontaneous activity was not altered by 250 R. WB dose delivered at a rate of 6 R./min.

(Rosenthal & Timiras, 1963). Amplitude increases and slight frequency decreases were observed during the first week and 35 days later following 500 R. exposures at a rate of 15 R./min. (Timiras, Woolley, Silva, & Williams, 1967).

BRAIN CHEMISTRY RADIATION STUDIES

Radiation has been shown to influence vascular tissue (Russell, Wilson, & Tansley, 1949; Gerstner & Kent, 1957; Scholz, Schlote, & Hirschberger, 1962; Haymaker, 1962; McDonald & Hayes, 1967; Redmond, Rinderkneckt, & Hudgins, 1967; Roizin, Machek, Liu, Caveness, & Carsten, 1968), white matter (Clemente & Holst, 1954; Brownson, Suter, & Diller, 1963; Zeman, 1966; Schjeide, Yamazaki, Haack, Ciminelli, & Clemente, 1966), RNA and DNA (Caster, Redgate, & Armstrong, 1958; Yamanoto, Feinendegen, & Bond, 1964; Hallen, Hamberger, Rosengren, & Rockert, 1967; Lloyd, Nicholson, & Peacocke, 1967; Ordy, Samorajski, Horrocks, Zeman, & Curtis, 1968), with only slight evidence for influence directly on neurons (Arnold, Bailey, Harvey, Haas, & Laughlin, 1954).

Serotonin or 5-hydroxytryptamine (5-HT) may be involved with the chemoprotective mechanism of irradiated animals (Brinkman & Veninga, 1962). Pretreatment with 5-HT increased the survival time of male mice receiving between 542-880 R. WB irradiation (Doull & Tricou, 1961). Intraperitoneal injections of ^{14}C-labelled 5-HT produced little radioactivity in the brain following irradiation. Precursors such as

tryptophan and 5-hydroxytryptophan were taken up by the brain tissue (Streffer, 1966). Interference with the pituitary-adrenal system by an adrenalectomy did not alter the radioprotective effect of 5-HT (Streffer, 1967; Bulat, Supek, & Deanovic, 1966). Protective injections of mercaptoethylguanidine produced no change in the serotonin levels of the brain following irradiation (Ershoff, Hellmers, & Wells, 1962). Injections of radioprotectors, cysteamine and cystamine, depleted the neurosecretory cells in both the hypothalamus and posterior pituitary of normal nonirradiated rats. After irradiation the protected animals had very little cell depletion and abundant material was present in both the cytoplasm and axons (Duchesne, Hajdukovic, Beaumariage, & Bacq, 1968).

An increased supply of tryptophan metabolities in the urine of mice after irradiation has suggested a general disturbance in the ultilization of tryptophan (Melching, 1968). The general disturbance as measured in terms of 5-HT brain content following irradiation has produced conflicting results. Little or no change in 5-HT content of whole brain or brainstem tissue has been reported immediately with doses below 1.2 kr. (Ershoff & Gal, 1961; Randic, Supek, & Lovasen, 1962; Bulat et al., 1966; Stransky, 1966; Komesu & Haley, 1968) and at doses of 4.5 kr. (Speck, 1962). Decreases in whole brain and hypothalmus content have been reported in the 500-10,000 r. range (Renson & Fischer, 1959; Ershoff et al.,

1962; Speck, 1962; Nair, 1965, 1967; Ordy et al., 1968) and when irradiated animals were compared to ad libitum fed controls (Ershoff & Gal, 1961). Irradiation in vitro at the highest dose in this range has produced a drop in serotonin levels in the thalamus and an increase in the pons-medulla regions (Lott & Hines, 1968). An increase in the medulla area has been reported 72 hours following irradiation in vivo (Nair, 1965). Increases in serotonin brain content have been reported with doses of 8.0 and 1.2 kr. (Randic & Supek, 1962; Palaic, Randic, & Supek, 1963; Bulat et al., 1966; Nair, 1967; Egana & Velarde, 1967).

An increase of serotonin content with large irradiation doses suggested leakage in the "blood-brain" barrier. Thus far, the efforts to verify such a leakage with a simultaneous change in brain serotonin have gone unrewarded (Bulat et al., 1966; Streffer, 1966).

The reason for the conflicting results from the studies reporting serotonin content following irradiation has not been ascertained. Data reporting serotonin increases have used both bioassay and fluorometric techniques. Other factors such as the specific tissue assayed, time passage of the extraction procedure following irradiation, species, sex, age, temperature, handling procedures of the animals, and dose rate may account for the variance in content level (Kato, 1959; Welch, 1967).

Other substances have been known to change serotonin

levels by interfering with serotonin metabolism. The most commonly referred to substances are iproniazid and reserpine. The actions of these two substances seem to be different. Iproniazid, by inhibiting the activity of monoamine oxidase (MAO) seems to prevent the destruction of free serotonin. Reserpine may effect a rapid release of the bound serotonin; subjecting it to rapid destruction by MAO (Page, 1958; Harper, 1967; Kety, 1967a). Pretreatment with either iproniazid or reserpine did not interfere with the increases in 5-HT content following a 8 kr. dose. Preliminary experiments suggested the increase in 5-HT may be a consequence of increased 5-hydroxytryphan decarboxylase activity (Palaic et al., 1963).

Yet, electrophysiological effects have been noted in the prepyriform cortex with doses of reserpine and following 500 R. irradiation. The EEG pattern following the administration of reserpine in nonirradiated animals has been one of increased frequency and amplitude reduction. Following irradiation, reserpine had the opposite effect; it increased amplitude without altering frequency (Timiras et al., 1967).

Pharmacological changes following irradiation have been noted. The potentiation of barbiturate hynosis by serotonin (Page, 1958) has been observed following 5 and 10 kr. doses (Nair, 1965, 1967). Doses of barbituates have been less lethal in irradiated animals than in controls (Barnes, 1967). Barbituates have decreased the occurrence of the sinusoidal

waves and wave frequency, and increased the waves from the prepyriform cortex (Timiras et al., 1967). Stimulants have been found to have lethal effects in irradiated animals with the exception of strychnine which produced no differences between control and irradiated animals (Barnes, 1967). The EEG pattern from the prepyriform cortex following a subconvulsant dose of pentylenetetrazol and 500 r. irradiation was one of increased wave occurrence and amplitude (Timiras et al., 1967).

Changes in brain norepinephrine levels following irradiation have not been extensively investigated. Ordy et al. (1968) determined norepinephrine content (μg/brain) and concentrations (μg/g) following deuteron irradiation and found both these measures were unchanged following total doses of 500, 5000, and 10,000 r. The direction of the nonsignificant changes tended to be positive for 500 and 5000 r. doses and negative for the 10,000 r. dose.

Serotonin has been considered to be a chemical transmitter of the central parasympathetic system and norephrinephrine, the central sympathetic transmitter (Brodie & Shore, 1957). Localized concentrations of these substances seem to indicate their distribution within the CNS is similar with the highest concentrations in the diencephalon and mesencephalon (Bradley, 1968). The distribution of 5-hydroxytryptophan decarboxylase parallels that of serotonin except in the pyriform cortex and amygdala. The function of this enzyme is to

catalyze the decarboxylation of 5-hydroxytryptophan to yield 5-HT and carbon dioxide. Most of the serotonin in the rat's brain is bound to mitochondria (Page, 1958).

As suspected transmitter substances, both serotonin and norepinephrine seem to have excitatory and inhibitory effects (Bradley, 1968). Evidence has been presented for inhibitory effects of serotonin in some limbic structures (Eidelberg, Miller, & Long, 1966; Eidelberg, Goldstein, & Deza, 1967) and inhibitory effects on behavior (Sheard, 1969). Indications of serotonin as a transmitter substance have included the increases in brain serotonin following electrical stimulation (Breitner, Picchioni, Chin, & Burton, 1961), the iontophoretic blockage of serotonin by LSD (Boakes, Bradley, Briggs, Dray, 1969), and serotonin's depressive effect on evoked potentials (Pineda & Snider, 1963). Synaptic events have been implicated in irradiation studies. Synaptic delay has been reduced following 250 and 500 R. doses (Timiras et al., 1967). As stated on a different conceptual level, the role of norepinephrine in the brain has been one of mediating alertness, wakefulness, pleasure, euphoria, anger, and fear (Kety, 1967b). Serotonin has also been implicated in the role of emotions and functioning of the intellect (Woolley, 1967).

BEHAVIORAL RADIATION STUDIES

Assessment of irradiation on behavior has been complicated by abscopal and motivational changes which may inhibit

performance. Radiation induced alterations in physiological and sensory systems may become limiting factors in the organism's capacity to perform. Gastrointestinal (Eldred & Trowbridge, 1953; Hunter, Munson, Brown, & Abbatt, 1957; Zsebok & Petranyi, 1964; Kawakami, Negoro, & Kimura, 1968), cardiovascular (Brooks, 1956; Hunt & Kimeldorf, 1963; Phillips & Kimeldorf, 1963), respiratory (Brooks, Richey, & Pickering, 1957), thermoregulatory (Meredith & Finnegan, 1962), and sensory systems (Brown et al., 1955; Lipetz, 1955; Veninga, 1961; Bachofer, 1962; Devi et al., 1968; Cooper, 1968) have been affected by irradiation.

Another obstacle is that different species may not exhibit identical behavioral reactions despite comparable neural damage. The obstacle may, however, become an advantage. Assessment in a behavioral task may be a sensitive index of radiation changes since the responses in the animal's repertoire were utilized in the design of the task for that particular species. Reactions to irradiation have appeared to differentiate species at higher doses. Sharp epileptoid seizures of rabbits, immediate ataxia of rats and mice, prolonged muscular rigidity and vomiting of dogs, absence of convulsions in 100 kR. exposures of hamsters, vomiting in primates--all have been designated as characteristic reactions of species following exposure. HO exposures seem to be as effective as total body in producing neurological symptoms, thus leading to the concept that damage to the

CNS was the basis for the incapacitation (Kimeldorf & Hunt, 1965; Ross et al., 1954).

One of the most common and reliable signs of radiation sickness during the first week of exposure has been anorexia. Anorexia, in the present instance, is defined as an abnormal reduction in food intake under ad libitum feeding conditions. The degree of anorexia seems to be dependent on the species and the radiation dose. Rats and rabbits have reacted with complete abstinence of food, whereas guinea pigs have shown only a slight transient effect when comparable mortality doses were given (Smith & Tyree, 1954; Newsom & Kimeldorf, 1960). Primates appear to react promptly following sublethal total body exposures (Hunter et al., 1957). Higher doses generally have produced an extension of the anorexic period. The anorexic period may be avoided in rats by limiting the caloric intake prior to and after irradiation (Carroll & Brauer, 1961). Exposure of the gastrointestinal tract does not seem to be necessary for the appearance of anorexia.

One of the first behavioral indices which demonstrated a change following exposure to ionizing radiation was general activity. The general activity level was reduced during the first few days after exposure. Activity partially recovered to prior levels, only to be reduced again. The severity of the second depression was proportional to the dose and occurred within 10-15 days after exposure. A gradual return of normal activity followed the second depression in surviving

animals (Jones, Kimeldorf, Rubadeau, Osborn, & Castanera, 1954; Jones, Kimeldorf, & Rubadeau, 1955). The activity of rats living in spring cages was similar to that of activity wheels (Kimeldorf & Hunt, 1965).

Gastrointestinal changes parallel the activity reduction. Low abdominal doses have not produced a reduction in activity whereas nearly lethal doses have produced the effect. HO irradiation has produced the effect. The activity level of nonirradiated animals that were pair-fed the same amount as the head irradiated animals increased at the same time as the level of the head irradiated group. The second depression in activity level did not occur in the nonirradiated pair-fed control (Jones, Kimeldorf, Osborn, Castanera, & Rubadeau, 1957). Whole body irradiation has reduced the effect of a CNS stimulant (D-amphetamine) on activity levels (McDowell, Ziller, & Krise, 1967). Injections of bone marrow suspension in irradiated animals prevented the second activity depression (Jones, Kimeldorf, Castanera, Rubadeau, & Osborn, 1957).

Other species have exhibited a different pattern of activity following irradiation. After midlethal exposures of hamsters and guinea pigs, the initial drop in activity of these animals was limited to the first day. The second depression was more extended in these animals than in the rat (Castanera, Jones, & Kimeldorf, 1959).

Activity reduction simultaneously with anorexia was noted in monkeys during the second week following HO exposures

to 1.5 and 2.0 kR. The animals lost 17 to 35 per cent of their body weight by the end of the third week. General improvement began during the fourth week and by the end of the second month full recovery had occurred. Monkeys receiving 3.0 kR. HO irradiation showed no change in activity during the first 10 days. Thereafter the activity levels gradually diminished during the remainder of the three week survival period. HO doses of 4.5 and 6.0 kR. produced a decrease in spontaneous activity and an unwillingness to eat or drink two to four hours later. Within 55 hours 12 of the 16 animals were dead or killed when moribund, the other four survived from 5 to 12 days (Ross et al., 1954).

Swimming has also been used as a test of endurance for rats following irradiation. Irradiation depressed performance, with severity, but not duration, of depressed performance related to the dose (Kimeldorf, Jones, & Castanera, 1953). Preirradiation fatigue minimized the lethality of the swimming task following irradiation (Brown & White, 1958).

In swimming situations as well as other situations, the enforced performance may require a high level of physical effort and a capacity to cope with physiological stresses. The cost to the irradiated animal may be more than he can afford. Frequent sexual activity during the first 30 days after irradiation tended to increase the mortality of male mice (Rugh & Grupp, 1960). Exposures to temperatures of 6-10° C. and prolonged hypoxia increased the mortality of

rats (Kimeldorf & Newsom, 1952; Newsom & Kimeldorf, 1960). Monkeys receiving cumulative doses of 50 R. every two weeks spent more time supporting themselves on a pole above a pan of cold water than control animals (Harlow, 1962).

Ad libitum reductions in food intake and body weight loss seem to closely parallel activity changes and running speed during the first postirradiation week (Fields, 1957). Response rates on a VI-2 schedule decreased during the first postirradiation day for animals exposed to 300 and 500 R. doses and remained low through the third day for animals in the 500 R. group (Jarrard, 1963).

Other studies indicated the motivational fluctuations may be related to total dose. Rats receiving 1.2 kR. WB irradiation avoided the goalbox containing food in a T-maze, but not the rats receiving 400 R. doses (White & Brown, 1959). After receiving 2.5 kR. doses animals more frequently crossed a shock grid barrier to reach food than control animals in a 20 to 100 day period. Animals exposed to 5.0 kR. made fewer crossings for food and water than controls. Still, other studies have indicated irradiation has no effect on the instrumental and maze learning situations (Arnold, 1962).

Divergent results may stem from the behavioral technique itself. The problems designed for detecting changes in learning and retention may not be sensitive enough to detect radiation effects (Urmer & Brown, 1960) or the behavioral

procedures are not standardized (Kaplan, 1962). By examining the ways irradiation injury have been manifest, the behavioral situations might be designed to detect sensitive changes. Early disturbances which have been attributed to CNS damage have included those which involved movement, activity and visual survey (Davis & McDowell, 1962). The differentiation in performance between irradiated and control groups on such tasks involving activity wheels, spring suspended cages, and swimming may be explained on the basis of these early changes. Enhanced performance on delayed response and discrimination problems as a result of reduced general activity and distractibility may be explained on the same basis (Riopelle, Grodsky, & Ades, 1956; Harlow & Moon, 1956; Brown & McDowell, 1962). Other evidence has shown monkeys tend to display less other animal visual activity (Harlow, 1962) and exhibit longer latencies in opening home cage windows which extended their view (Arnold, 1962). Focal X-ray beams directed toward the frontal lobe have produced hyperactivity and when directed toward frontal parietal regions have produced more visual exploratory behavior (Davis & McDowell, 1962). Different deficits on the Wisconsin General Test Apparatus have been found with impairment in the frontal and parietal regions (Harlow, Schiltz, & Settlage, 1955). Persistent individual patterns in an insoluble maze problem (Murphree, Pool, & Frost, 1961) and decrements in the acquisition of an alternating pattern of immediate and

delayed reward (Ingersoll, Carsten, & Brownson, 1967) may be related to early irradiation changes.

On the other hand, the latency between irradiation and histological changes in the brain seem to be extended. Short postirradiation times revealed no changes with discrimination problems, whereas a 323 day postirradiation interval did (Arnold, 1962). Retention of locomotor activity has been impaired during the first day of a five consecutive day testing period each month for a 16 month postirradiation period. Maze exploration deficits occurred during each of the five day monthly testing periods. Both locomotor and maze exploration deficits were evident only after the second month of irradiation (Ordy et al., 1968). HO exposures of 2.0 kR. produced deficits on a discrimination problem with multidimensional cues 2.0 to 3.5 months following exposure. Deficits have been found two to five years after WB exposures (Riopelle, 1962).

Outside of curiousity and manipulatory drives in primates, the detection of behavioral changes following irradiation has been confined in adult animals to the study of the hunger and thirst drives. Some investigations have demonstrated disturbances in maternal behavior following exposures during gestation (Furchtgott, 1956, 1963). Alternations in self-stimulation rates of both high and low rate animals have been demonstrated during the first day of WB exposures (Haley, Bach-y-Rita, & Komesu, 1961).

Probably the most popularized of all behavioral irradiation studies are those concerned with conditioned aversion. Hardly a research oriented behaviorist is not familiar with irradiation induced saccharin aversion in rats (Garcia, Kimeldorf, & Koelling, 1955), although he may be less familiar with the Kool-Aid aversion in monkeys (Harlow, 1962). The saccharin conditioning studies involved low doses and contained a critical control group which was irradiated but did not drink saccharin during exposure. In contrast to the conditioned animals, the control animals drank large quantities of saccharin flavored water during postirradiation tests. The aversion has been established with trace conditioning procedures, in different species, and opthalmectomized rats (Garcia, Kimeldorf, & Hunt, 1961). The conditioned aversion to saccharin flavored fluid may be produced by providing a taste experience with saccharin in the post-anesthesia-postirradiation period, and in the shield member of a parabiotic rat pair united by skin vascular anatomosis (Hunt & Kimeldorf, 1967; Hunt, Carroll, & Kimeldorf, 1968).

Irradiation has served as an US in spatial avoidance (Andrews & Peterson, 1962). In the role of the CS, irradiation has aroused normal sleeping rats and opthalmectomized rats (Hunt & Kimeldorf, 1962, 1963), produced EEG desynchronization and habituation of the EEG activation pattern (Garcia et al., 1963; Garcia & Buchwald, 1963), and produced conditioned aversion (Garcia et al., 1962).

As mentioned earlier, detection of low doses was more easily obtained when a focal beam was directed in the vicinity of the olfactory bulbs (Garcia et al., 1964). Conditioned aversion to saccharin solutions and conditioned suppression responses have not been obtained in rats following destruction of the olfactory bulbs (Brust-Carmona et al., 1966; Dinc & Smith, 1966) or nasal occlusions (Riege, 1968) and in monkeys following bilateral sectioning of the olfactory tracts (Taylor, Smith, Wall, & Chaddock, 1968).

CHAPTER III

METHODS AND PROCEDURES

SUBJECTS

The subjects were 17 mature, mongrel, male cats whose body weight varied from 2.0 to 6.5 kg. at the onset of the experiment. Preliminary study was performed with two electrode implanted animals, PR-3 and PR-4. These animals received total doses of 1000 r. and 800 r., respectively. For the major investigation the electrode implanted animals were divided into three total dose groups. The three groups consisted of animals receiving 2000 r. HO doses (IE-3, IE-15, and IE-16), 3000 r. HO doses (IE-4, IE-12, and IE-14), and 500 r. WB doses (IE-7, IE-8, IE-9, and IE-10). Two electrode animals, IE-6 and IE-17, served as control animals. Another group of animals, IA-1 and IA-3, were not implanted with electrodes nor trained to perform the behavioral task, but received 3000 r. HO doses. IA-2 was a control animal for this group.

The cats were obtained from the city pound and housed in individual stainless steel cages in the air-conditioned animal colony of the Science and Research building at the University of Houston. The animals received feline distemper and pneumonitis vaccine injections upon their arrival and an additional distemper injection one week later. The animals

remained isolated from the other animals in the colony for at least two weeks.

ELECTRODES

The electrodes were constructed from 30 gauge nichrome, alloy wire which was coated with formvar. The wire was obtained from the Driver Harris Company and had a resistance of 6.68 ohms per foot.

To construct parallel bipolar electrodes wire lengths of approximately six inches were initially cut and stretched to increase tautness and decrease the probability of bending. A predetermined millimeter length was measured on two strands of wire and the wires were bent at a right angle at this length. One strand of wire was clipped and filed until it was approximately 1.5 millimeters shorter than the tip of the other wire. The two strands of wire were cemented together in a parallel arrangement along the premeasured distances with epoxylite (The Epoxylite Corporation) and oven dried. The insulation was scraped for a distance of one millimeter from the tips of the wires with a separation in the vertical axis of approximately one millimeter. The electrodes were checked with an ohmmeter and normal saline solution.

The insulation was scraped about three millimeters distance from the tip of the electrode leads and gold pins (Amp No. 202124-2) were crimped on the scraped tips. A small amount of dental cement was applied to the bend of the two

wires for clasping the electrode into the carriage of the stereotaxic instrument.

SURGERY

The operating table and all surgical instruments were washed with an all purpose cleaner and sterilized either by autoclaving for 30 minutes at 250° F. or soaked in 1.750 solution of zephiran chloride overnight.

Prior to surgery each animal was weighed and the anesthetic, sodium pentobarbital (1 gr./cc.), was injected into the liver or peritoneal cavity. One cc. of anesthetic for each five pounds of body weight was considered a safe dosage. Each animal was given 1 cc. of Coaglin (Pioneer Veterinary Supply, Inc.) to prevent excessive blood loss and 1.5 cc. of crysticillin to combat infection.

The locus of the incision was shaved with animal clippers and washed with phisohex and alcohol. Two cc. of 2.5% procaine hydrochloride solution and 1 cc. of adrenalin chloride were injected subcutaneously in the area to serve as a local anesthetic and to reduce loss of blood.

The animal was placed in a Stoelting stereotaxic instrument and a medial sagittal incision approximately three inches in length was made to expose the cranium. The periosteum was scraped laterally to expose the underlying bone and the head muscles surrounding the incision were removed. Gauze sponges soaked in normal saline solution were placed subcutaneously

on either side of the exposed cranium. The cranial surface was washed with normal saline and dried with sponges.

The skull was marked bilaterally with food coloring. Using the Horsley-Clarke method and the coordinates of Reinoso-Suarez (1961), the cortical and subcortical coordinates were: A-P -10.0, L 5.0, for the visual cortex; A-P 2.0, L 9.0, V 5.0, for the hippocampus; A-P 11.5, L 9.5, V -5.0, for the basolateral amygdala; A-P 12.0, L 3.5, V -2.0 for the lateral hypothalamus; A-P 23.0, L 7.0 for the motor cortex; and A-P 31.0, L 2.5, for the olfactory bulb.

The animals PR-3 and PR-4 used in the preliminary study had the same electrode placements except one electrode was implanted ipsilaterally in the lateral hypothalamus and the posterior hypothalamus (A-P 10.0, L 1.2, V -4.0) and bilaterally in the auditory cortex (A-P 3.0, L 15.5).

Each of the loci was trephinated with a dental burr (No. 3). Each electrode was placed into the electrode holder on the stereotaxic carriage and positioned over the locus of trephination, and then lower to a predetermined depth. Dental cement was applied to the skull to secure the electrode in position.

The gold pin leads were inserted into a microminature 50 lead terminal (Amp No. 202121-1) in a predetermined order with a special tool (Amp No. 126031-2-0). The terminal with attached leads was secured to the skull with a keyhole configuration in the frontal sinus and a 20 gauge stainless

steel loop in the posterior region of the skull. The area beneath the terminal was covered with dental cement.

The incision around the terminal was washed with normal saline solution and sutured. Pelizone (National Laboratories) was applied topically to the locus of the incision to prevent surface infection. Each animal was permitted a two week surgical recovery period before training began on the behavioral task.

Systematic histological verification of the accuracy of the electrode placements is still in progress.

BEHAVIORAL APPARATUS

The testing chamber, liquid feeder system, photic stimulator, control and timing apparatuses have been described elsewhere (Grandstaff, 1965; Uzzell, 1967), but a brief description will be given.

The experimental chamber was positioned in a shielded room before a one-way vision glass through which an animal during experimental sessions could be viewed from the adjacent room. Constructed of formica and plexiglas, the chamber had inside dimensions of approximately 48 x 30 x 30 inches and was divided into two small compartments by a clear plexiglas partition. Only one of the small compartments was used during behavioral task performance. Bright ceiling illumination was provided by dc connectors to a 50W 30V bulb enclosed in a clear glass case. Dim illumination was provided in the

adjacent small compartment by a 50W 30V bulb which was enclosed in a painted glass case.

A Grass model PS 2 stimulator activated a photic light which was mounted externally on one side of the chamber. The stimulator was calibrated to produce 7 cps. flashes. The flashes were seen through a port at one end of the small chamber. The intensity setting of the photic stimulator was 2.0.

Directly below the photic port in the small chamber was a polyethylene tube and feeding cup. Milk reinforcement was delivered into the cup from a liquid container by closing a relay which delivered 24V to a solenoid valve (Skinner Electric Valve Division). The valve which was externally mounted on one side of the chamber above the photic light had a $\frac{1}{4}$ inch ortifice and a rating of 10 Psi. Operation of the solenoid valve was accomplished by the animal pressing a bar located two inches lower and to the right of the feeding cup, or externally by the experimenter. Behind a second port which was located directly above the photic port was an audio speaker. The speaker was connected to an oscillator and an auditory amplifier.

An eight channel, one inch, perforated, mylar tape was fed through a Rheem tape reader (Model No. RR-301RB) which was a photocell tape reader with a reading speed of a line per 0.1 second step. The function of the circuits of the tape reader was to convert the information in the form of

perforations on the tape into electronic signals. The perforations were recognized by means of photoelectric cells as the tape passed under the reading lamp. The output from each cell was synchronized with the feed hole track so that all outputs appeared simultaneously. The output of each photocell was amplified and available for external routing and use.

Foringer and Grayson-Stadler control and timing equipment were used in conjunction with the Rheem tape reader to program the desired stimulus inputs and contingencies into the experimental chamber. Digital counters cumulatively recorded the responses and stimulus events.

ELECTRICAL RECORDING APPARATUS

A seven channel Grass electroencephalograph (Model III) was the basic recording unit. Electrical brain activity could be recorded with all seven pens. Two binary pens which had been added to the writer unit provided recordings of the animal's bar presses and the onset and duration of the photic stimulus event.

A 50 lead cable from the electroencephalograph was connected by a conduit to two junction boxes mounted in a small compartment above the experimental chamber. The first junction box received connections for leads 1 through 24 and the second box received connections for leads 25 through 50. Connections from the junction boxes comprised the cable to which the animal was attached.

The cable was about three feet in length and contained 50 leads, each of which had 3 millimeters of insulation scraped from the tip and gold pens (Amp No. 202123-2) attached. The gold pens were inserted into a 50 lead male terminal (Amp No. 12603-2). The terminal was constructed to be attached to the female receptacle chronically mounted on the cat's head. The cabling was a microminature, coaxial, steel center cable (Microdot Inc.) which reduced self generated noise by more than a factor of 100 to 1.

The cable placed through a metal and plexiglas sleeve was inserted through a port in the ceiling of the experimental compartment. A spring was wound around the lower end of the cable to prevent wear from raising and lowering the cable. The tension on the cable was sustained by a balanced tension system mounted above the experimental chamber.

In addition to paper EEG recordings, the electrical brain activity and event information were magnetically recorded on two tracks of $\frac{1}{4}$ inch tape at 7 ips with an Ampex recorder (PR-10). The onset and offset of the recorder for each trial were automated.

The magnetic recording system was a multiplex one, capable of recording seven EEG channels of analog information, eight channels of binary data, and one time reference channel. All event channels plus analog channels 1, 6, and 7 were recorded on one tape track. Analog channels 2, 3, 4, 5, and the reference channel were recorded on the second tape track.

The analog channels were frequency modulated in a range of 3.9 to 14.5 kc. Stimulus and behavioral events were amplitude modulated. Signals from the EEG were fed into a system of analog clippers which removed extraneous transients from the EEG and fed the signal into the frequency modulators.

RADIATION SOURCES

The animal irradiation was performed with the assistance of personnel from the Radiological Safety Office and the Radiation Biology Laboratory at Texas A. & M. University. The radiation sources utilized were Co^{60} with gamma energies of 1.17 and 1.33 Mev.

Two radiation facilities were utilized for the experiment. The first consisted of an 800 Curie source contained in an 18 inch diameter lead shield with an irradiation port of 6 inches in height and $3\frac{1}{2}$ inches in width. Animals PR-4, PR-3, IE-3, and IE-4 received HO exposures from this facility. The animals were exposed in a radiation field of 90 r./min. Total exposures were determined by exposure time with animals PR-4 receiving 800 r., PR-3 receiving 1000 r., IE-3 receiving 2000 r., and IE-4 receiving 3000 r.

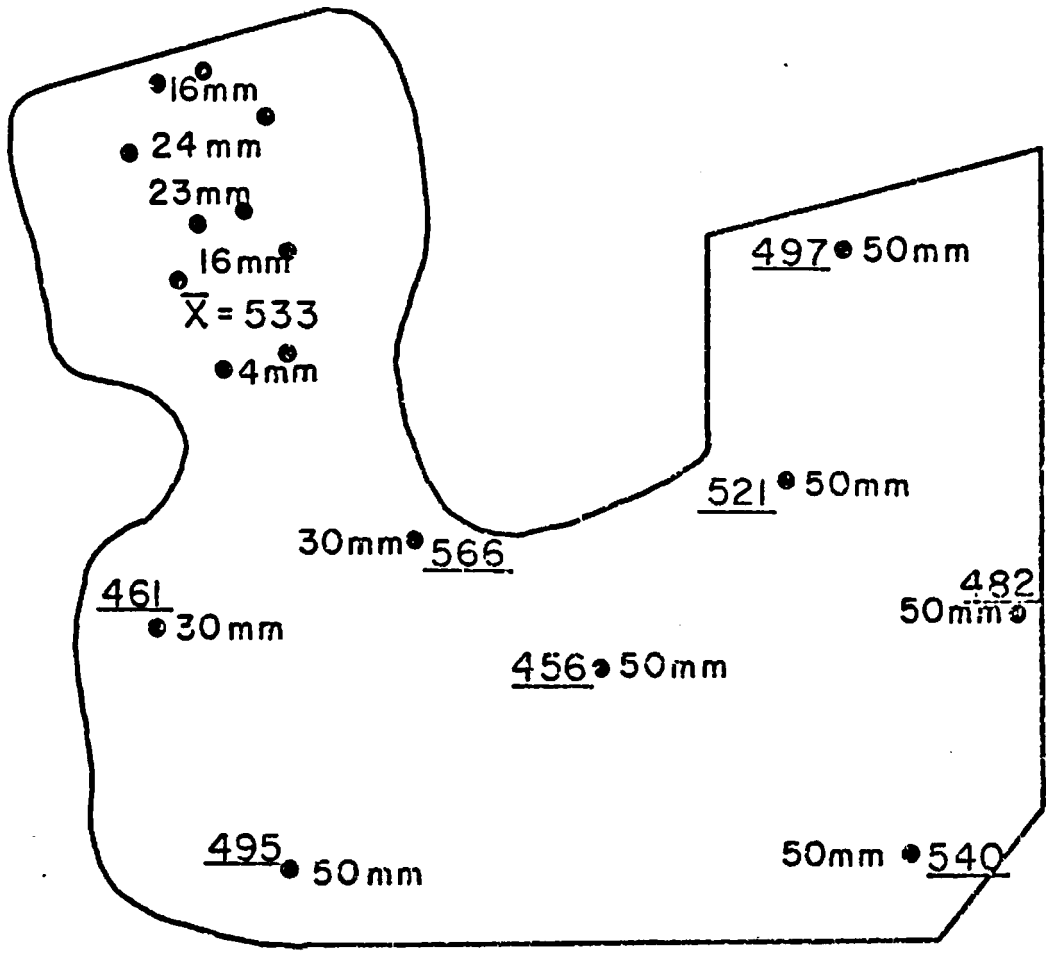
A Picker Model C-10,000 Co^{60} teletherapy unit was utilized for animals IE-15, IE-16, IE-12, IE-14, IA-1, IA-3, IE-7, IE-8, IE-9, and IE-10. The unit housed a 3400 Curie Co^{60} source and had the advantage of an adjustable collimator. Thus the field size could be adjusted at a fixed distance

from the source which enabled the HO and WB exposures to be performed in the same geometry, at the same dose rate and exposure time. Animals IE-15 and IE-16 received 2000 r. to the head, while animals IE-12, IE-14, IA-1, and IA-3 received 3000 r. to the head. A WB exposure of 500 r. was given animals IE-7, IE-8, IE-9, and IE-10. The dose rate for the Picker unit exposures was 60 r./min.

DOSIMETRY

The dosimetry involved the use of two separate systems, the Victoreen R meters and Harshaw LiF-7 thermoluminescent dosimeters (TLD). The Victoreen R meters were certified as calibrated against the NBS standards. Depth dose determination was accomplished by implanting TLD dosimeters in a paraffin phantom. Dosimetry for the experimental work had an accuracy of $\pm$ 10%. The paraffin phantom was exposed twice with dosimeters implanted at various depths and locations. The total time of the first exposure was 10 minutes with a mean total dose of 515.57 $\pm$ 37.4 r. Figure 1 shows the first phantom which had a total mean dose for the head area of 533 r. and the total doses and dosimeter depth of the other body regions. Figure 2 shows the second phantom with dosimeters implanted at approximately the same depths as the locations of the head electrodes. The exposure time was the same as the first phantom and the mean total dose for the phantom was 498.00 $\pm$ 21.17 r. The total dose varied with the

CAT PHANTOM I



WHOLE BODY — GAMMA IRRADIATION

Fig. 1. Whole body exposure of a paraffin phantom showing mean total dose for the head and total doses for other body regions and dosimeter depths.

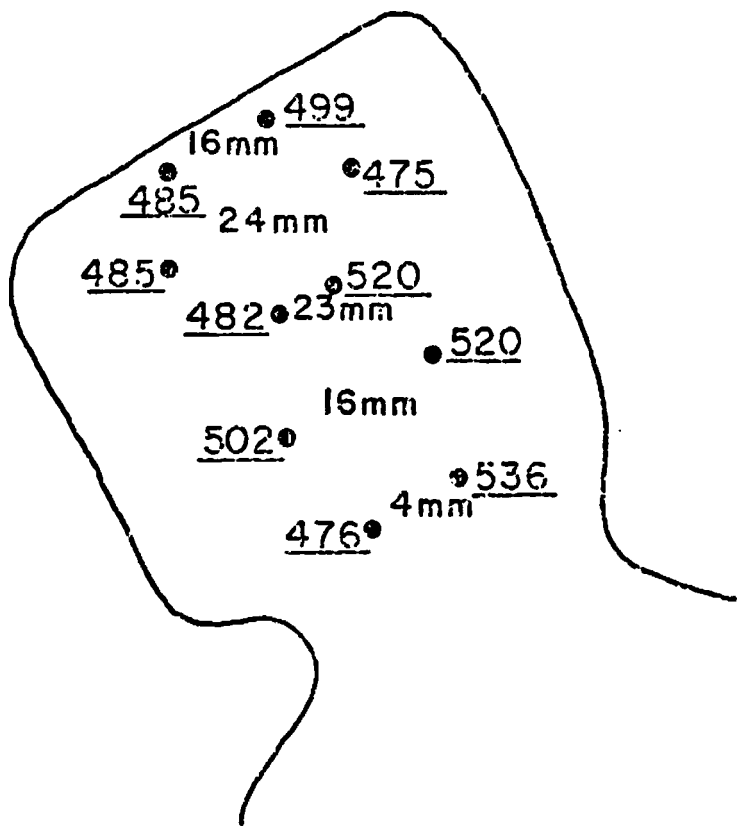


Fig. 2. Head only exposure of a paraffin phantom showing total doses and dosimeter depth which approximate the depth of implanted electrodes.

depth of the dosimeter. Total doses decreased as the depth of the dosimeter increased.

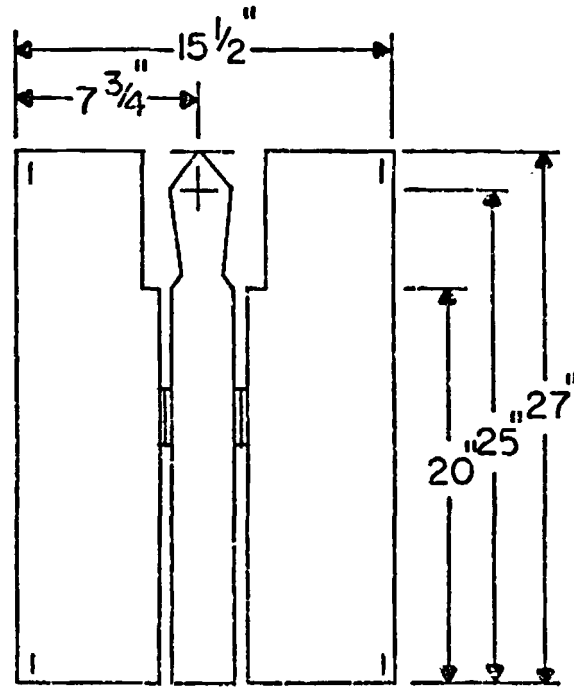
RESTRAINING DEVICES

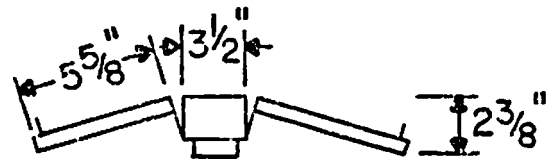
The HC irradiated animals were positioned on the restraining device as shown in Figure 3. The head was located in the region of the + shown in Figure 3. The head was stabilized with masking tape attached to the device.

The WB animals were placed in a 10.25 x 10.25 x 7.50 inch cardboard box. The area in the box was small, and although some movement was possible by the animals, it was assumed to be negligible.

BODY WEIGHT PROCEDURE

On the day of surgery and each morning at the same time thereafter the animals were weighed, and food intake, water intake, general health, and behavior were noted. A weight loss occurred following surgery, but by the end of the two week recovery period, most of the animals' weights were at presurgical levels. During the third week following surgery each animal was weighed and the ad libitum food and water intake was recorded. During the fourth week following surgery, a 30 minute feeding period was instituted for each animal in his home cage. The policy was believed to be necessary to adjust the animals to a limited feed schedule and to gradually reduce body weights for task motivational





ANIMAL RESTRAINING DEVICE

Fig. 3. A restraining device used for the head only exposed animals.

purposes. During preliminary training, the animals were maintained at 85-90% of their mean body weights. The mean body weight was determined from the weights of the animals during the third postsurgical week, or when the daily body weights did not deviate more than ± 0.1 kg. for one week. The animals' body weights were maintained at 95% of the mean body weight, once the behavioral criterion of the task had been achieved.

Each animal received a 30 minute feeding period in his home cage following daily preliminary training periods and later performance sessions on the behavioral task. The diet consisted of Purina cat chow and water, which was supplemented by the milk the animal received during the task performance. During preliminary training, when a more stringent body weight was necessary for task motivational purposes, the available water in the home cage was reduced, or not given. Food was always available during the 30 minute feeding period in the home cage following task performance. The quantity of food was varied to maintain a certain percentage of body weight.

PRELIMINARY TRAINING PROCEDURE

All training and task performance was done at the same time each day. During the fifth postsurgical week, the method of successive approximations was used to shape a bar pressing habit for milk reinforcement in each animal. During

the preliminary training, a small plexiglas box which limited the cat's movement was inserted into the chamber. Bright illumination was used in the chamber and the milk reinforcement consisted of one 13 oz. can of evaporated milk mixed with water to make a total volume of one liter. A small amount of milk was available in the feeding cup prior to the animal's introduction into the chamber. A 400 cps tone accompanied the milk dispension, either when the animal pressed the bar, or when the experimenter externally closed the circuit to the solenoid valve.

After the animal was able to press the bar on a continuous reinforcement (CRF) schedule during a 30 minute period, the schedule of reinforcement was changed as follows: Day 1) 10 minutes of performance on a CRF schedule, followed by 20 minutes on a FR schedule of three bar presses for each reinforcement; Day 2) 10 minutes on a CRF schedule, followed by 10 minutes on a FR-3 schedule, and 10 minutes on a FR-5 schedule; Day 3) 10 minutes on a FR-3 schedule, followed by 20 minutes on a FR-5 schedule; Day 4) 30 minutes on a FR-5 schedule. After completing the series of schedules, the animal was shifted to Phase I training.

PHASE I TRAINING PROCEDURE

All trials during Phase I were completely automated by the Rheem tape reader and auxiliary equipment. Phase I consisted of a 50 trial session with a 10 second reinforcement

period indicated by a 7 cps. flashing light (S^+) during each trial. During the 10 second period, each bar press was reinforced by 0.3 cc. of milk; all other bar presses within a trial were not reinforced. The trials were presented on a VI schedule with an average trial of 30 seconds. The training sessions began with the presentation of a S^+ period.

A reset period of 10 seconds just prior to the onset of the S^+ period was introduced on the second training day. If an animal made more than a specified number of responses during the reset period, a timer reset and the 10 second period began again, delaying the S^+ period until the animal did not exceed the designated number of responses. On the second day of Phase I training, the designated number of responses was five. On the third day and subsequent days, the number of responses was three.

Since the transition from the preliminary training to Phase I training was sometimes difficult for an animal, a method was found which reduced the abruptness of the transition. The method consisted of the experimenter manually stopping the Rheem tape reader during an S^+ period, and thus providing a slow, responding animal more time to obtain the reinforcement. Otherwise, the animal sometimes extinguished his responses when reinforcement was not available and never performed the task. The method was terminated when the animal began to respond more during the S^+ period.

At the conclusion of each daily session, a record was

kept of the following: 1) total number of responses during the 50 trial session; 2) total number of reinforced responses (S_R^+); 3) total number of intertrial responses (ITR) or errors; and 4) a ratio of ITR/S_R^+. A ratio value of one, meant the animal was responding the same during the intertrial and S^+ periods. A ratio value of less than one, meant the animal was making more responses during the S^+ period than during the intertrial interval. The animals continued training on Phase I until they reached a ratio value of less than one during three consecutive 50 trial sessions.

Following criterion performance on Phase I, EEG paper records and magnetic tape recordings were collected from five of the daily sessions during behavioral performance of the task. These records were collected on alternate days without the 10 second reset period. Alternate days were chosen to prevent performance deficits. The reset period was eliminated because computer analysis of the EEG recording was based on a fixed time period. The reset period extended a trial for a variable amount of time depending on the behavior of the animal.

When the five preirradiation recordings were collected, each animal was taken to Texas A. & M. University for the irradiation procedure. An EEG recording was collected on the same day, approximately three hours after irradiation. Subsequent EEG recordings were collected at each 24 hour period for at least the next eight days of all animals that were not sacrificed for brain assays.

EEG ANALYSIS PROCEDURE

All recordings were played back on an Ampex (PR-10) at 7 ips through a multiplex demodulator system which separated the carrier frequencies from analog and event signals by band pass filters. The EEG signals were then available for computer analysis. The event signals from the tape recording triggered the computer.

The main function of the hybrid analog computer was to average the voltage in different frequency bands during a specified time period (Benignus, 1967). The computer has been described elsewhere in terms of control and analog computing components (Sheer, 1970). The computer could analyze two different EEG signals simultaneously and through the A-D conversions of the digital voltmeter (3440A Hewlett-Packard) display the two digital records. Both the digital records were displayed on paper tape (Model 1404, Systron) and chart paper (Mark 280, Brush Instruments).

Six computer programs were prewired into Amp program boards. Program board one was selected for the present analysis. In order to simplify the EEG analysis process, the following discussion will consider only the procedures for analysis of one EEG signal. The procedure for the analysis of the second EEG was the same and occurred simultaneously with duplicate equipment.

The high pass filters were adjusted until the lower

frequency half power point was 12 cps. The demodulated EEG signal was fed into the system from the tape recorder and the signal was adjusted until it did not exceed the capacity of the machine, ± 10V. The output of each of the five band pass filters with frequencies centered at 20, 25, 31.5, 40, and 50 cps. were adjusted until they did not exceed ± 10V. Each frequency band had a ± ½ range of 23% of the centered frequency. The sum of the five frequency bands, denoted as summing broad band, was adjusted until it did not exceed ± 10V. A 9.7 second epoch was chosen and the digital timers were set for a pre-epoch analysis time, a delay period of event information, and a stimulus and post-epoch periods.

The digital voltmeter was adjusted to an automatic 100 V. and the sample rate was set on hold. Thus, a three digit accuracy was permitted. The pen sensitivity of the Mark 280 was set at 200 mV./line and the paper speed was set at 10 mm./sec. The tape recorder was started slightly before the beginning of the first trial to be analysed. Since the analysis was automated from that point on, only the paper tape and chart paper were moderated.

The values on the paper tape and the heights of the bars on the chart paper records represented relative power values for the five band power centered frequencies during three different 9.7 second time periods of each trial. To determine these values from the EEG signals, the absolute voltage outputs of each of the five band pass filters were fed into

squaring operators and then stored in integrators as mean absolute power for the time period. Simultaneously the sum of the squared outputs of the five frequencies was stored. Since absolute power was not considered stable across animals and electrodes, the relative contribution of each of the five frequency bands was determined by dividing the mean absolute power of each band pass filter by the sum of the five frequency bands. These values were converted from analog to digital values and were displayed numerically on paper tape and as spectrograms on chart paper.

BRAIN ASSAY PROCEDURE

Brain sections from three electrode animals (IE-3, IE-4, and IE-16) and two nonelectrode animals (IA-1 and IA-3) that had received 2000-3000 r. HO irradiation were assayed for serotonin and norepinephrine content. The controls for the assay included the two electrode control animals (IE-6 and IE-17) and one nonelectrode animal (IA-2). The animals were killed by decapitation at either 3 hours, 24 hours, and 8 days following irradiation.

The brain tissue was removed as rapidly as possible and frozen for analysis at a later time. The brain tissue for the assay included the olfactory bulbs and a temporal lobe section which was dissected between stereotaxic coordinates: 8-17 A-P; 4.5 L; and 0.0 V. The temporal lobe section was included because it contained the amygdala which receives tracts from the olfactory bulb.

The procedure for the brain assay was developed at the Texas Research Institute of Mental Sciences, and consisted of a modification of spectrophotofluorometric procedures (Bodganski, Pletscher, Brodie, & Udenfriend, 1956; Shore & Olin, 1958; Udenfriend, 1962; Wise, 1967a, 1967b).

The fluorescence spectra was obtained with an Aminco-Bowman spectrophotofluorometer (No. 4-8202, American Instrument Co.). The unit included an optical system, a dc power supply, and a photomultiplier microphotometer. The xenon lamp (No. 416-992) had starting and operational circuits to the dc power supply and an output voltage of 19.8 dc. The photomultiplier microphotometer was ac powered, and included an electronic chassis, control panel, indicating meter, and a 1P 28 photomultiplier tube. The photomultiplier tube had a violet maximum response limit at 650 mμ. Spectra were read from the meter indications on the photomultiplier microphotometer. The number five slit arrangement was used for both serotonin and norepinephrine determinations. A polarizer (Kodak polar screen, No. 5) was used in the number four slit position for the serotonin determinations.

Deionized distilled water passed through a demineralizer and reagent grade chemicals and solvents were used. All pipettes and culture tubes were washed in Calgonite. Weekly standard stock solutions were made from 0.01 N hydrochloric acid combined with serotonin creatinine sulfate monohydrate or l-noradrenalin bitartarate (Regis Chemical Co.), and stored in the refrigerator.

The N-butanol was washed with 10% volumes of 1.0 N sodium hydroxide, 1.0 N hydrochloric acid, three times with distilled water, and finally with 0.01 N hydrochloric acid. The N-butanol was checked with pH paper in the 1-3 range for a pH of 2. Reagent grade heptane was washed with 10% volumes of 1.0 N sodium hydroxide, 1.0 N hydrochloric acid, and twice with distilled water. A 1M acetate buffer solution was prepared from 2M acetic acid and 2M sodium acetate solution. The pH was adjusted to 5.0. Each month a 0.1 N iodine solution was prepared with absolute ethanol, and a 0.05 N sodium thiosulfate solution was prepared with distilled water.

The brain tissue was weighed and homogenized with a motor (Black & Decker) driven teflon pestle in a pyrex homogenizing tube and four parts of .01 N hydrochloric acid. A 1.5 to 3.0 aliquot (depending on the total volume) was transferred to a 50 ml. culture tube containing 3 g. of salt. To this mixture, 25 ml. of butanol was added. An internal standard of 1μg. was prepared by adding the standard solutions of serotonin and norepinephrine to a tube containing the homogenate from a control animal. The tubes were placed on an automatic shaker for one hour and then centrifuged for 15 minutes at 1500 rpm. From each tube a 20 ml. supernatant aliquot was placed in another 50 ml. culture tube containing 3 ml. of .01 N hydrochloric acid and 25 ml. of heptane. These tubes were placed on the automatic shaker for five minutes, after which they were centrifuged for 3 minutes at

1000 rpm. The aqueous phase was carefully removed with a 10 ml. pipette. A 1 ml. aliquot of the aqueous phase from each of the 50 ml. culture tubes was transferred to two small culture tubes for determinations of serotonin and norepinephrine content.

For the serotonin determinations, 0.3 ml. concentrated hydrochloric acid was added to the aqueous phase, slightly agitated, and the contents of the sample tube poured into a quartz cuvette. The activation wavelength was 295 mμ and the fluorescence wavelength was 540 mμ. For determining the fluorescence of a blank, 1 ml. of 0.01 N hydrochloric acid plus 0.3 ml. concentrated hydrochloric acid were placed in the cuvette. The readings of the internal standard and the blank were used in the calculations of extracted serotonin.

The final procedure for the norepinephrine determinations consisted of adding 1 ml. of acetate buffer and 0.1 ml. of iodine to the aqueous phase. After six minutes 0.2 ml. of sodium thiosulfate was added. Finally, 4 ml. of alkaline-ascorbic-acid-EDA solution was added to the aqueous phase. The alkaline-ascorbic-acid-EDA solution was prepared just prior to its use. The quantity of the preparation varied with the number of sample tubes for norepinephrine determinations. The solution was prepared in the following proportions: nine parts of 5.0 N sodium hydroxide added to 0.2 ml. EDA, followed by 1.0 ml. of 2% ascorbic acid.

Following the addition of the alkaline-ascorbic-acid-EDA

solution, a 30 minute time period was allowed before sample readings were made. A blank was similarly prepared for norepinephrine by taking 1 ml. of 0.01 N hydrochloric acid and adding the acetate buffer. The order of the addition of sodium thiosulfate and iodine was reversed for the blank, before adding the alkaline-ascorbic-acid-EDA solution.

At the end of the 30 minute period, the contents of each sample tube were poured into a quartz cuvette. The activation wavelength for norepinephrine determinations was 400 mμ and the fluorescence wavelength was 500 mμ.

CHAPTER IV

RESULTS

In an effort to determine the initial effects of irradiation on the electrical, chemical, and behavioral aspects of the present study, the data from the early time periods following irradiation were selected for comparison with pre-irradiation data. One later time period just before the animals became moribund, was chosen for comparison purposes. The time periods included: 3, 24, 48 hours and 7 to 8 days. The animals were placed into three groups based on area of exposure. These groups included the 500 r. WB animals, 2000-3000 r. HO animals, and the controls. The 2000 and 3000 r. HO animals were combined into one group since the data collected from these animals did not appear to be distinctively related to total dose.

The data from these three groups of animals during the specified time periods will be presented in the following sections: 1) EEG recordings of the olfactory bulb and computer analysis; 2) serotonin and norepinephrine content of some olfactory structures; 3) behavioral task data and general observations.

EEG RECORDINGS OF THE OLFACTORY BULB AND COMPUTER ANALYSIS

EEG recordings were not obtained from all six animals in the 2000-3000 r. HO group during the specified time periods.

One animal was sacrificed for olfactory tissue assay after the 3 hour EEG recording was made. Two other animals were sacrificed for similar reasons after 24 hour EEG records were obtained. The fourth animal was sacrificed after 8 day EEG recordings were obtained. The last two animals were sacrificed after 15 and 17 days.

Figure 4 shows an EEG recording from the right olfactory bulb of IE-16, an animal in the 2000-3000 r. HO group. The upper part of Figure 4 shows the olfactory bulb recording during a nonstimulation portion of a trial on the behavioral task at the preirradiated, 3 hour, and 8 day time periods. The lower part of the figure shows the olfactory bulb recording during the presentation of the visual stimulus at the preirradiated, 3 hour, and 8 day time periods. The nonstimulation and stimulation portions of the trial seemed to have no appreciable effect on the olfactory bulb recordings. The 40 cps. spindle bursts which are characteristic of the bulb, were present during preirradiation periods. EEG recordings of the olfactory bulb at the 3 hour period showed a reduction in amplitude and a loss of the 40 cps. bursts. The effect was transitory; and the spindle burst and amplitude of the 8 day recording was similar to the preirradiated record. The effect was present in the records of other animals in the HO group.

In comparison, Figure 5 shows an EEG recording of the right olfactory bulb of IE-8, an animal in the 500 r. WB

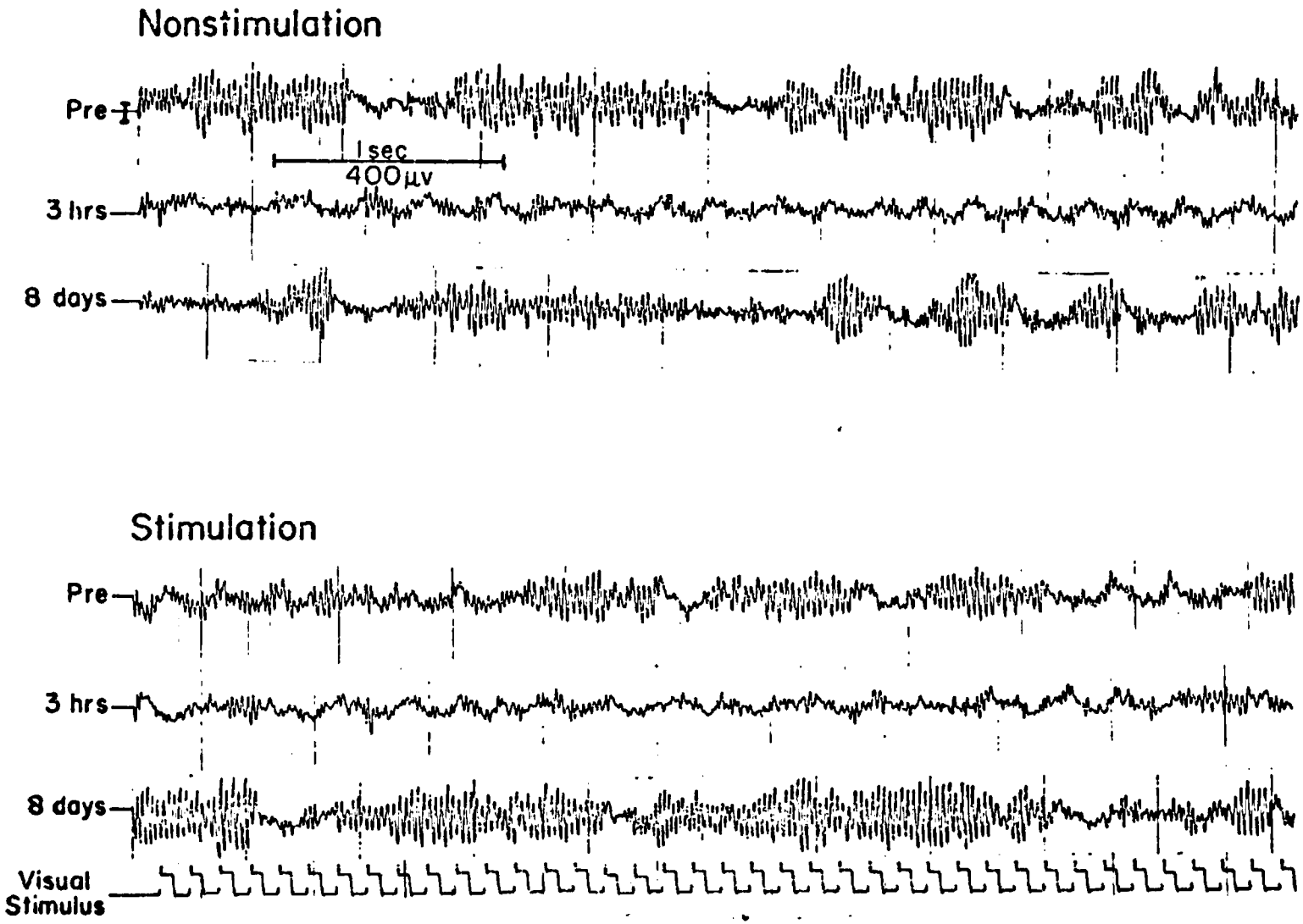


Fig. 4. Olfactory bulb recording of a head only irradiated animal (IE-16) during nonstimulation and stimulation portions of a trial at preirradiation, 3 hour and 8 day postirradiation periods.

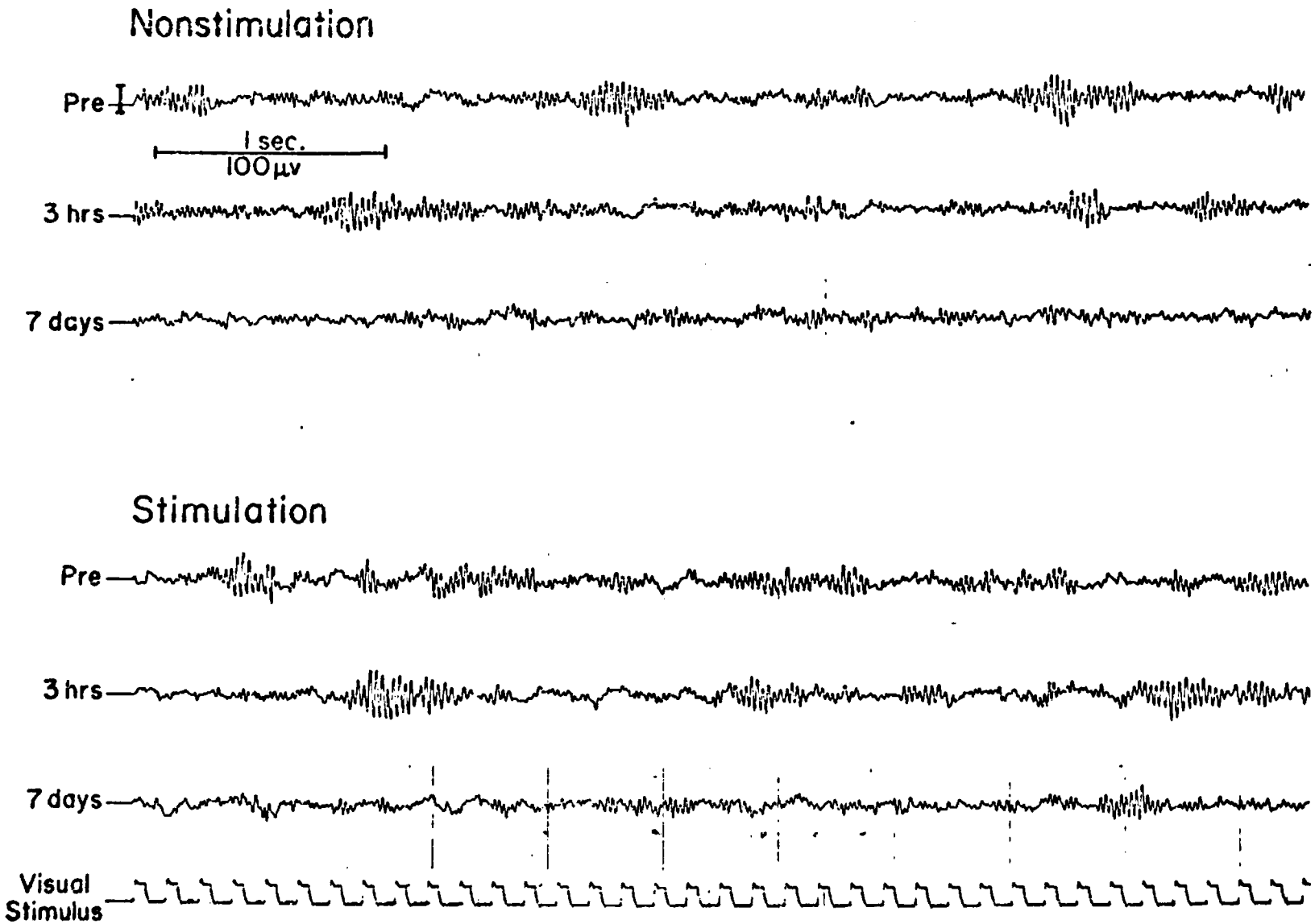


Fig. 5. Olfactory bulb recording of a whole body irradiated animal (IE-8) during nonstimulation and stimulation portions of a trial at preirradiation, 3 hour and 8 day postirradiation periods.

group. Again, the stimulation period had no effect on the olfactory structure. The 3 hour recording seemed to be unchanged in comparison to the preirradiated recording. The recording of the olfactory bulb at 7 days showed a loss of the 40 cps. and a slight amplitude reduction. The changes in the recording of the WB animal at 7 days was similar to the one which occurred in the record of the HO animal at the 3 hour period.

Similar olfactory bulb recordings were obtained from the other three animals in the WB group. Records obtained after 7 days showed the gradual deterioration associated with morbidity and death in the WB animals. The olfactory bulb changes in the WB group at 7 days were not transitory. One animal in the WB group died 12 days following irradiation. Another animal was sacrificed during terminal stages 10 days following irradiation. The other two animals in the group were sacrificed during morbidity 14 days following irradiation.

A recording from the left olfactory bulb of IE-17, a control animal is shown in Figure 6. The recording seemed to be unchanged during preirradiation, 3 hour, and 8 day periods. The olfactory bulb changes seemed to be genuinely related to irradiation and not to other extraneous factors which might occur in transporting the animals to and from the Co^{60} source.

To add confirmation to the changes in the 40 cps. activity noted in the olfactory bulb recordings, the recordings

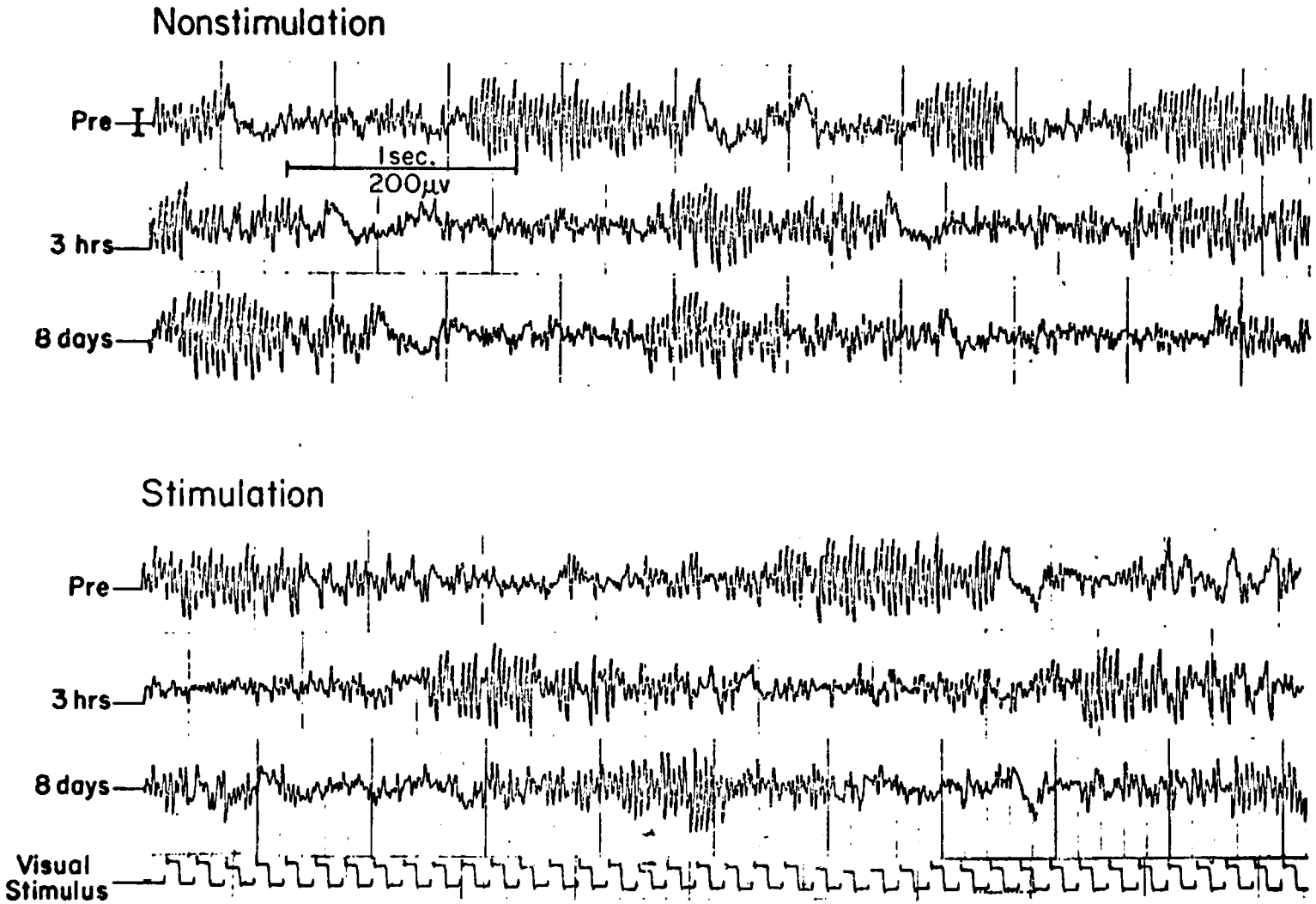


Fig. 6. Olfactory bulb recording of a control animal (IE-17) during nonstimulation and stimulation portions of a trial at preirradiation, 3 hour and 8 day postirradiation periods.

were computer analysed. The relative amount of power from the 40 cps. band pass filter was selected from the last 10 trials during three preirradiation sessions, and 3, 24, 48 hour, and 7 or 8 day sessions. The relative 40 cps. power from the HO and WB groups were averaged and the standard deviations were computed for the time periods during the 10 second nonstimulation and stimulation portions of the 10 trials. These values are depicted in Tables 1, 2, 3, and 4.

A Wilcoxon Signed Rank test was applied to relative amounts of 40 cps. in the HO group during the preirradiation and 3 hour time periods and preirradiation and 24 hour periods after the estimation of two missing data points (Winer, 1962). The estimation of the missing data points was required to obtain a sample size large enough for the tabled probabilities of the Wilcoxon test. The two missing data points occurred during one 3 hour session when recording equipment failures precluded the EEG record collection, and during a 24 hour period because an animal had already been sacrificed for brain tissue assay. The Wilcoxon values obtained from comparing the preirradiation time with the 3 and 24 hour periods were significant at the .05 level in both olfactory bulbs during nonstimulation and stimulation portions of the trial.

Statistical tests were not possible in the HO group during the 48 hour and 8 day postirradiation periods because of the reduction in sample size. Animals had been sacrificed

TABLE 1

MEAN PERCENTAGE AND STANDARD DEVIATIONS OF 40 CPS. EEG ACTIVITY IN THE RIGHT OLFACTORY BULB DURING 10 NONSTIMULATION PERIODS FOR HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Group		Nonstimulation				
		Pre	3 Hours	24 Hours	48 Hours	7-8 Days
HO 2000-3000 r. (N=6)	Mean	34.9	26.0*	28.5*	36.8[a]	39.1[a]
	SD	15.8	11.2	13.4	14.6	20.8
WB 500 r. (N=4)	Mean	47.2	35.6	37.6	37.4	33.5*
	SD	14.5	18.4	17.5	18.0	6.5
HO 1000 r. (N=1)	Mean	41.6	41.1	43.2	43.8	36.9
HO 800 r. (N=1)	Mean	64.0	64.6	64.1	64.5	65.7
Control (N=1)	Mean	67.0	67.4	69.1	67.9	67.0

[a]The values are based on three animals.

*$p < .05$

TABLE 2

MEAN PERCENTAGE AND STANDARD DEVIATIONS OF 40 CPS. EEG ACTIVITY IN THE RIGHT OLFACTORY BULB DURING 10 STIMULATION PERIODS FOR HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Group		Stimulation				
		Pre	3 Hours	24 Hours	48 Hours	7-8 Days
HO 2000-3000 r. (N=6)	Mean	34.0	27.6*	27.2*	39.1[a]	39.7[a]
	SD	17.7	14.2	13.7	22.2	23.0
WB 500 r. (N=4)	Mean	44.0	33.4	37.0	35.2	32.8*
	SD	13.5	16.9	17.2	15.9	6.3
Control (N=1)	Mean	52.3	54.1	54.9	55.3	53.6

[a]The values are based on three animals.

*$p < .05$

TABLE 3

MEAN PERCENTAGE AND STANDARD DEVIATIONS OF 40 CPS. EEG ACTIVITY IN THE LEFT OLFACTORY BULB DURING 10 NONSTIMULATION PERIODS FOR HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Group		Nonstimulation				
		Pre	3 Hours	24 Hours	48 Hours	7-8 Days
HO 2000-3000 r. (N=6)	Mean	50.6	34.2*	44.9*	49.0[a]	47.6[a]
	SD	15.0	10.3	13.1	11.8	18.0
WB 500 r. (N=4)	Mean	47.7	42.4	44.0	44.3	36.3*
	SD	6.3	8.8	3.6	7.7	7.9
HO 1000 r. (N=1)	Mean	26.0	21.6			
Control (N=1)	Mean	62.7	63.7	63.1	62.8	63.4

[a]The values are based on three animals.

*$p < .05$

TABLE 4

MEAN PERCENTAGE AND STANDARD DEVIATIONS OF 40 CPS. EEG ACTIVITY IN THE LEFT OLFACTORY BULB DURING 10 STIMULATION PERIODS FOR HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Group		Stimulation				
		Pre	3 Hours	24 Hours	48 Hours	7-8 Days
HO 2000-3000 r. (N=6)	Mean	45.3	32.0*	37.4*	46.5[a]	47.2[a]
	SD	13.4	9.9	13.7	16.3	18.0
WB 500 r. (N=4)	Mean	45.6	38.0	42.3	41.9	36.3*
	SD	6.3	8.0	9.1	5.3	9.1
Control (N=1)	Mean	43.9	43.9	44.7	44.8	44.7

[a]The values are based on three animals.

*$p < .05$

for brain tissue assay during the 3 hour and 24 hour periods. The values at the 48 hour and 8 day postirradiation periods represented the mean percentage values of 40 cps. in the olfactory bulbs of three animals. Inspection of the values in Tables 1, 2, 3, and 4 indicated the values at the 48 hour and 8 day time periods were relatively unchanged from the preirradiated value of the HO group.

The percentage of 40 cps. activity in the olfactory bulb of the WB group were compared by a two way classification test (Wilcoxon & Wilcox, 1964) which assumed the samples had been drawn from the same population as given by Friedman (1937). Comparison of several treatments which were repeated under different conditions with a control condition was possible with the test. In the case of the present data, the percentage of 40 cps. in the olfactory bulb was compared during 3, 24, 48 hour and 7 day periods with the preirradiation value. Tables 1, 2, 3, and 4 show a significant reduction in 40 cps. activity in both olfactory bulbs during nonstimulation and stimulation portions of a trial at the 7 day period.

Control percentage values of the 40 cps. olfactory bulb activity were determined for one animal. The other control animal was sacrificed after the 24 hour recording session. During a preliminary investigation with two animals that received 800 and 1000 r. HO doses, the relative power of the 40 cps. activity in the olfactory bulbs did not seem to

change, except slightly in the right olfactory bulb of the 1000 r. HO animal at the 7 day period. The values of the 800 r. animal did not seem to vary. Both of these animals were considered to be further control animals during non-stimulation periods. Recordings from the 800 and 1000 r. animals were made during their bar pressing performance for milk reinforcement on an FR-5 schedule.

SEROTONIN AND NOREPINEPHRINE CONTENT

Two sample tubes obtained from the olfactory tissue of each animal were tested for the serotonin and norepinephrine content. One sample was available from the olfactory tissue of each control animal since the second control tube served as an internal standard.

The calculations for content were based on the internal standard. The meter readings of the blank tube were subtracted from all sample readings, control readings, and the internal standard. The control meter readings was subtracted from the internal standard. The subtracted value represented 1μg. of known serotonin and norepinephrine and was used in the calculations of the content in the sample readings. Sample content was obtained by proportion (1μg/meter reading of known content = X/sample meter reading). Control values were obtained by the ratio of control meter readings to the meter reading of known content.

The assay procedure was originally based on a gram of

tissue in each sample tube. In the present procedure the brain weight had been diluted by a greater factor than the original procedure and various size aliquots of homogenate had been used at the beginning of the assay procedure. The sample and control values were corrected by a dilution factor of the brain weight in each aliquot of homogenate. These values along with the sample size and deviation measures are shown in Table 5. Comparisons of the values obtained in the present assay with other published values is not possible since the published values have not combined the tissue from the olfactory bulbs and the temporal lobe for content assay.

The results of the serotonin assay suggested a slight increase in the substance during the 3 hour period following irradiation of the HO animals. No change seemed to occur in the other time periods shown in Table 5 in comparison to the control value. The results of the norepinephrine assay showed a decline in the substance at the 3 hour period and a gradual return to the control values at the 8 day period.

BEHAVIORAL DATA

The Phase I task chosen for behavioral performance was rather a complex one. All animals were trained to a rather stringent criterion performance before preirradiation and postirradiation data were collected. In spite of the training the animals exhibited extreme variability, and the initial effects of irradiation were difficult to assess.

TABLE 5

CONTENT OF SOME OLFACTORY TISSUE IN 2000-3000 r. HEAD ONLY IRRADIATED AND CONTROL ANIMALS[a]

Substance	Postirradiation Time			
	Control	3 Hours	24 Hours	8 Days
Serotonin	.49±.040(3)	.60±.020(2)	.44±.020(4)	.42±.040(2)
Norepinephrine	.50±.080(3)	.36±.030(2)	.40±.007(6)	.42±.020(2)

[a]The values are expressed as average μg/g of wet tissue ± S.E. The number in parenthesis refers to the number of samples.

Median values of behavioral measures, however, will be presented.

Five preirradiated 50 trial sessions were selected for comparison with the 3, 24, 48, and 8 day postirradiation sessions. These were the five preirradiation sessions during which EEG recordings were collected from each animal. The five sessions were selected because the 10 second reset period was eliminated from the trials during these sessions, and that was the case of the postirradiation sessions. The reset period tended to reduce the number of ITR because frequent responding extended a time period without reinforcement, and the animal soon extinguished his responses. The performance of one of the HO animals deteriorated rapidly without the reset period during the daily postirradiation sessions. The animal's values were discarded from the HO group.

The behavioral measures chosen for each of the 50 trial sessions were: 1) number of total responses; 2) ITR; 3) S_R^+; and 4) the ratio of ITR/S_R^+. The average values of each animal's measures were obtained for the five preirradiation sessions. The preirradiation and the postirradiation medians of the measures for 3, 24, 48 hour and 8 day periods are shown for the HO, WS, and control groups in Table 6.

The reduction in the total number of responses during the 48 hour and 8 day sessions in the HO group seemed to be misleading. The medians at these time periods were based

TABLE 6

MEDIANS OF THE BEHAVIORAL TASK MEASURES OF HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Total Response

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HC 2000-3000 r. (N=5)	718	646	701	574[a]	253[a]
WB 500 r. (N=4)	518	354	435	481	398
Control (N=2)	397	482	375	448	485
	407	408	406		

ITR

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HC 2000-3000 r. (N=5)	227	184	207	146[a]	119[a]
WB 500 r. (N=4)	156	100	107	116	67
Control (N=2)	57	14	49	62	62
	97	97	81		

(Table continued on next page)

TABLE 6 (Continued)

S_R^+

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HO 2000-3000 r. (N=5)	482	462	489	427[a]	134[a]
WB 500 r. (N=4)	344	300	328	348	318
Control (N=2)	338	468	326	386	424
	310	311	325		

ITR/S_R^+

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HO 2000-3000 r. (N=5)	.45	.28	.42	.33[a]	.45[a]
WB 500 r. (N=4)	.48	.32	.32	.32	.16
Control (N=2)	.19	.03	.12	.16	.14
	.30	.31	.25		

[a]The values are based on two animals.

only on two animals. One of these animals showed an extreme reduction in total responses, whereas the other animal did not. Statistical evaluation was not possible because of the small sample size.

The medians in Table 6 indicate a reduction in total responses of the WB group at 3 hour and 8 day periods. As with all summary measures, the median value sometimes misrepresents the whole group. Only two of the four WB animals showed a reduction in total responses during the 3 hour and 8 day periods.

Comparisons between the preITR and the 3 hour ITR of the HO group suggested a decline in ITR at the 3 hour period. The change seemed to be rather meaningless since the ITR of one of the control animals showed a similar change. The effect would appear to be related to some other factor besides the initial effects of irradiation on task performance.

The ITR of the WB group showed a decline at the 3 hour period as well as the other groups. A decline in ITR was evident in the WB group at the 8 day period, and was considered a later effect associated with gastrointestinal injury. One of the animals in the WB group had completely stopped responding by the 8 day period and the responses of the other animals were considerably reduced.

The S_R^+ was unchanged in both the HO and WB groups except during the 8 day period of the HO group. Again the 8 day

median depicted in Table 6 was misleading since the value was based on two animals, only one of which showed a reduction in S_R^+.

The 3 hour ITR/S_R^+ of the HO group was reduced in comparison to the preirradiation value. The 8 day ITR/S_R^+ of the WB group declined in comparison to the preirradiation value. Both reductions were without consequence, since the ratio changes merely reflected the reduction in ITR at the same time periods.

The control medians shown in Table 6 appeared to be relatively stable, except for the 3 hour and 8 day periods. During both these time periods, one control animal increased his total responding and reinforcements.

Although the behavioral observations and symptoms of radiation sickness were not systematically recorded each day, it seemed meaningful to disclose the general behavioral changes noted following irradiation. Most of these changes appeared at later time periods. The reactions of the HO and WB groups to irradiation did not seem to be similar, and they appeared at different postirradiation time periods.

No reactions to irradiation appeared in the HO group during the first 24 hours. Mydrasis occurred at 48 hours in the three HO animals that were not sacrificed at earlier time periods for tissue assay. Dark discolorations appeared about 5 days after irradiation on the skin near the mouth, chin, and forepaws, and in one animal in the HO group, on the skin

near the eye. These discolorations became ulcers during the 7-9 day period. Blindness, as judged by the failure to avoid obstacles, occurred in the three animals during the 7-9 post-irradiation day period. Mucous secretions from the mouth, which suggested bronchial or respiratory infections, appeared during the 7-9 postirradiation day period.

Food intake began to decrease in the HO group during the 8 day as shown in Table 7. The trend continued in one animal until he was sacrificed after 15 postirradiation days. Another animal began to eat normally after 15 days and continued to do so until he was sacrificed after 17 days. The third animal was sacrificed 8 days after irradiation. Body weight loss began to appear 8 days after irradiation (see Table 7) and continued. Water intake which was not presented in the table showed a similar trend. No change in water intake occurred until the 8 postirradiation day. After that time, the animals began to drink more water in their home cages since they were no longer responding for milk reinforcement in the behavioral task. No change was noted in the animals' behavior toward the experimenter. The animals demonstrated their usual affectionate reactions when they were removed from their home cages and eagerly followed the experimenter to the behavioral task chamber even though they were not performing the task in later days.

The animals in the WB group developed no radiation symptoms or behavioral changes initially, such as the mydrasis

TABLE 7

MEDIANS OF FOOD CONSUMPTION AND BODY WEIGHT OF HEAD ONLY, WHOLE BODY, AND CONTROL ANIMALS

Food Consumption (g.)

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HO 2000-3000 r. (N=5)	41	41	51	40[a]	28[a]
WB 500 r. (N=4)	30	30	30	30	16
Control (N=2)	37	33	33	36	36
	26	20	18		

Body Weight (kg.)

Group	Postirradiation Time				
	Pre	3 Hours	24 Hours	48 Hours	8 Days
HO 2000-3000 r. (N=5)	3.69	3.85	3.86	3.97[a]	3.83[a]
WB 500 r.	3.78	3.76	3.69	3.70	3.61
Control (N=2)	4.21	4.22	4.19	4.23	4.20
	2.62	2.67	2.64		

[a]The values are based on two animals.

that appeared at 48 hours in the HO animals. Changes in the WB group were noted about 6-8 days after irradiation in three animals in the WB group. The animals became somnolent and excessive sleep periods were noted. The symptoms were noted in the fourth animal of the group at a later time period, 10 days after irradiation. The animals showed gradual deterioration in the days following the excessive sleep periods. Neurasthenic symptoms appeared. No emetic symptoms were present, but a mucous secretion was present in the anal region. The animals would frequently attempt to defecate the secretion. A foul odor was associated with the presence of the animal.

Food intake of the WB group began to decrease as shown in Table 7 during the 8 day period. The water intake increased about the same time since the animals were not performing the behavioral task for milk reinforcement. Body weights began to decline 8 days after irradiation as shown in Table 7. The animals in the WB group during later periods greeted the experimenter affectionately, but could not follow her to the behavioral task chamber. In an attempted walk, the animals moved very slowly and lethargically. They would frequently stop, then attempt to walk further until they stopped and could not move.

CHAPTER V

DISCUSSION

The present study had three aspects: electrical activity of the olfactory bulbs, serotonin and norepinephrine content of the olfactory structures, and behavioral performance on a task which involved olfactory cues. The main objective was to investigate the effects of gamma irradiation on these aspects.

The principal finding of the present study was a reduction in the characteristic 40 cps. activity in the olfactory bulb following irradiation. The effect appeared 3 and 24 hours following HO irradiation, and 7 days following WB irradiation. Other published spectrum analyses of olfactory activity following irradiation were not available for comparison with the major finding. Certainly frequency changes have been noted in olfactory areas, such as the prepyriform cortex, following irradiation (Timiras et al., 1967) and suggested by the loss of olfactory spindles with continuous irradiation of extremely high doses (Cooper, 1969). The responsiveness of single olfactory units to low doses of irradiation have suggested the sensitivity of the area (Cooper & Kimeldorf, 1966, 1967; Cooper, 1968). In the present study, a single, acute, 2000-3000 r. HO dose seemed to be effective in reducing the 40 cps. spindles through the 24 hour period after irradiation. Lower HO doses of 800 and

1000 r. were ineffective. A threshold effect of total doses seemed to occur, beyond which irradiation changes were initially manifested.

What changes incurred by irradiation would result in the 40 cps. loss in the bulb at 3 and 24 hours, and no loss at 48 and 8 days? Surely direct injury to a cell body, dendrite, or axon would not be alleviated 48 hours after irradiation. Nerve tissue does not seem to respond to injury in that manner. One suggestion is a neurochemical change, since such a change could occur within the time span of the electrical changes. Of course the roles of serotonin and norepinephrine as neurotransmitters have been speculative (Bradley, 1968). In the present investigation increases in serotonin and slight decreases in norepinephrine were found in olfactory tissue 3 hours after irradiation. The lack of increased serotonin during the 24 hour period may be due to the inability to detect very slight changes with the assay procedure. Other researchers have noticed an increase in serotonin whole brain content following irradiation (Palaic et al., 1963; Egana & Velarde, 1967).

Altered permeability in the blood-brain barrier has been suggested for the serotonin increase in the reported studies. But neither preirradiation injections of iproniazid and reserpine, nor postirradiation injections of serotonin has altered the serotonin content (Palaic et al., 1963; Bulat et al., 1966).

Other evidence for relating the loss of 40 cps. to increased serotonin content has appeared in studies which have injected precursors or blocking agents of the indolamines and catecholamines, and measured electrical brain activity. In normal cats, elevations of brain serotonin has been accomplished in two ways: by the administration of a serotonin precursor, 5-hydroxytryptophan (5-HTP) and a MAO inhibitor. The electrical activity in the olfactory bulb in both instances showed a marked depression in the 40 cps. spindles. The electrical changes did not seem to be related to the presence of the precursor. Blocking the enzyme which decarboxylates 5-HTP produced no change in electrical activity, even when 5-HTP was administered later (Eidelberg et al., 1966). Other substances such as pheniprazine, LSD, and harmine which have produced an elevation in brain serotonin, have also produced a depression of 40 cps. activity (Eidelberg, Long, & Miller, 1965).

The opposite EEG changes have occurred with a rise in norepinephrine content. The 40 cps. activity increased in the olfactory bulb following the administration of a precursor of norepinephrine. Depletion of norepinephrine produced a loss of 40 cps. spindles in the olfactory bulb (Eidelberg et al., 1966). The two substances seem to interact since depletion of brain norepinephrine has been associated with an elevation of serotonin concentrations (Reis, Miura, & Weinbren, 1967).

Respiratory arrest following large doses of serotonin has been accompanied by complete disappearance of evoked potentials. Reappearance of the evoked potentials has occurred soon after the administration of artificial respiration. Serotonin was suspected of acting as a chemical depressor of certain respiratory processes within the neuron, such as an increase of oxygen consumption (Pineda & Snider, 1963). The sensitivity of olfactory units to oxygen concentrations during low doses of irradiation has been demonstrated (Cooper et al., 1966; Cooper, 1970).

Another suggested function of serotonin has been made based on studies involving excessive amounts of the substance. Excess serotonin may block conduction at the synapse by inducing a persistent state of depolarization. The presynaptic impulses would be ineffective and would not evoke postsynaptic responses in such a situation (Brodie & Shore, 1957).

The 40 cps. loss might be accounted for in terms of chemical changes, but the question of peripheral effects must also be considered. Air flow through the nasal passages has been shown to be necessary for 40 cps. activity in the olfactory bulb (Sheer et al., 1963; Gault & Coustan, 1965). In the present study air flow was not interfered with directly. The different sensitivities of the olfactory bulb and the receptors following irradiation have been shown. Irradiation has abolished the 40 cps. olfactory spindles before the unit

responses of the bulb. Even after the olfactory unit responses were abolished by additional irradiation, the receptor responses and slow wave potentials of the olfactory mucosa were still functional (Cooper, 1969).

If the olfactory receptor were stimulated by ozone or some other radiation by product, some indication might have appeared in the control animals of Hull et al. (1965). In the present study, neither of the control animals showed any change in the olfactory bulb recordings. It does not seem likely that stimulation of a by product during the irradiation procedure would persist through the first 24 hours.

The electrophysiological changes seemed to be unrelated to behavior. In the present study no behavioral changes were noticed in the animals until 7 days. The general well-being of the animals seemed to be the same as before irradiation. Irradiation did not have a disruptive effect on task performance. No retention impairments were evident and each animal responded to the task characteristically. This is not to say that behavioral impairments might never occur following irradiation. If the time interval between irradiation and testing were lengthened, certain deficits might appear (Ordy et al., 1968). If the task were acquired after irradiation (Ingersoll et al., 1967), or if the task required locomotive behavior (Jones et al., 1954, 1955), or if the task were aversive (Harlow, 1962), impairments might appear. But in the manner performance was tested in the present study, no initial deficits appeared.

A dissociation between electrical activity of the brain and behavior has occurred after chemical injections. The 40 cps. spindles have been depressed or abolished when an animal was placid (after a-methyl-meta-tyrosine), restless (after pheniprazine), or mildly provoked to explosive rage (after LSD) (Eidelberg et al., 1965). Normal behavior has been present in rabbits when serotonin levels have been changed 20-30% of control values (Haggendal & Lindqvist, 1963).

The evidence for a relationship between the loss of 40 cps. activity and serotonin content completely unrelated to behavior seems tenable from the results of the HO irradiated animals in the present study. The evidence for the same relationship between 40 cps. loss and increased serotonin content in the WB irradiated animals seems less conclusive from the present results. No content analyses were performed on the brain tissue of these animals. The WB animals did not show a 3 and 24 hour 40 cps. loss in the olfactory bulb. Several reasons could account for the differences between the HO and WB groups. The WB animals did not receive as high a total dose as did the HO animals; nor was the beam focused as intensely in the head region. A smaller dose may have required a longer latency before the 40 cps. loss appeared. On the other hand, the loss of 40 cps. activity in the WB animals occurred during the initial stages of radiation sickness, a time when chemical changes as well as gastrointestinal

and vascular changes were occurring.

The results of the present study showed a loss of 40 cps. activity in the olfactory bulb at 3 and 24 hours following HO exposures to gamma irradiation which appeared to be related to increased serotonin content in these structures and not related to behavior. A similar 40 cps. loss occurred during the onset of radiation sickness, 7 days following WB exposures. The loss may or may not be related to increased serotonin content, although possibly the same process may account for the electrical change in both HO and WB exposures. Whether the process involves serotonin as an inhibitory neurotransmitter (Eidelberg et al., 1967), or as a chemical depressor in certain respiratory processes (Pineda & Snider, 1963) remains to be seen.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The initial electrical, chemical, and behavioral changes following gamma irradiation were investigated with five total dose groups of 17 mongrel, male cats that received either sham irradiation, 500 r. whole body (WB), 800 r. head only (HO), 1000 r. HO, or 2000-3000 r. HO irradiation. During preliminary investigation, 800 r. and 1000 r. HO irradiated animals were trained to perform 30 min. daily sessions on a FR-5 schedule for milk reinforcement and EEG recordings were taken during performance. For the major investigation, four 500 r. WB, six 2000-3000 r. HO, and two sham irradiated animals were trained to perform a visual discrimination task which required the animals to press a bar for milk reinforcement during the presence of a 10 sec. 7 cps. flashing light. Fifty daily trials were continued until the animals were making less than one intertrial response for every reinforced response as determined by a criterion ratio during three consecutive sessions. Total number of responses, intertrial responses, and reinforced responses were recorded following each daily session.

Following criterion performance, EEG recordings were taken on the unrestrained animals during five performance sessions on the task. The animals were exposed to Co^{60} sources at Texas A. & M. University. The dose rates for

these exposures were 60 and 90 r./min. Total doses were determined by exposure time. Subsequently 3, 24, 48 hour and 7-8 day EEG recordings were taken while the animals were performing the behavioral task. Six chronically implanted and three nonelectrode animals receiving 2000-3000 r. HO or sham irradiation were sacrificed at 3, 24 hours and 8 days for serotonin and norepinephrine assays of olfactory tissue.

The EEG recordings and computerized frequency analysis of the 2000-3000 r. HO animals showed a loss of the 40 cps. activity in the olfactory bulb 3 and 24 hours after irradiation during 10 sec. nonstimulation and stimulation periods of a trial. The loss of the 40 cps. activity appeared in the EEG recordings and computerized frequency analysis of the WB animals 7 days after irradiation during 10 sec. nonstimulation and stimulation periods of a trial. No 40 cps. changes were observed up to 7 days after 800 and 1000 r. HO irradiation. Serotonin content of the olfactory tissue tended to increase and norepinephrine content tended to decrease slightly at the 3 hour period following irradiation. No impairments in behavioral performance were detected until radiation sickness appeared.

The 40 cps. loss was attributed to increased serotonin content in the olfactory structures. Behavior as measured by performance on a visual discrimination task was unrelated to the initial electrical and chemical changes.

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