ELECTROPHYSIOLOGICAL AND BEHAVIORAL CORRELATES OF ELECTRICAL AND CHEMICAL STIMULATION OF THE BASAL AMYGDALOID COMPLEX

By

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INTRODUCTION

The two classical experimental methods for studying the relationship between brain and behavior have been to examine the effects of destroying or stimulating specified central nervous system areas. However, progress in understanding the electrical and biochemical aspects of brain activity has been limited by the insufficient specificity of such techniques for simulating excitation or inhibition of neural systems.

Unfortunately, it does not appear that the brain is made up of discrete localized areas of specific functions. For example, Wheatley (1944) once postulated that the medial hypothalamic region played a major integrative role for affective behavior. However, the medial hypothalamic area overlaps so extensively with hypothalamic feeding and drinking centers in all common laboratory animals, that lesions or electrical stimulation almost invariably induce concurrent effects on emotional behavior, food and water intake, sexual behavior, and self-stimulation pleasure centers (Miller, 1957, 1960; Olds, 1962; Teitelbaum & Epstein, 1962; Teitelbaum & Stellar, 1954; Wolfe, 1964).

Thus, it is evidence that a number of diverse motivational and emotional functions all involve this small area of the brain, and that lesions or electrical stimulation are relatively crude and indiscriminate in that they are likely to affect more than one of these diverse functions. When such diverse effects are produced from the same site, one response may obscure another. For example, stimulating the lateral hypothalamic region with

high intensity current produces frantic, escape-like activity which precludes the possibility of observing anything else. Furthermore, stimulation of specific sites in the hypothalamus elicits several effects in relatively unpredictable combinations (Robinson, 1964). One must also electrically stimulate a fairly large population of cells or fibers in order to produce observable behavioral effects (Miller, 1961).

The picture, then, is one of specific functional networks that are spread out and interlaced, rather than of functions that are anatomically separated into discrete areas. Such an overlap of anatomy necessarily makes investigation of the organization of the brain with lesions or electrical stimulation more difficult.

The situation is no less complex when the many extrahypothalamic areas that contribute to the regulation of the organism's emotional and physiological balance are considered. Even minute lesions or low-intensity electrical stimulation of the amygdaloid complex affect concurrently such emotional behavior as fear, defense, and aggression (Bard & Mountcastle, 1948; Schreiner & Kling, 1953) and often produce additional effects on food and water intake (Grossman & Grossman, 1963). Likewise, lesions or electrical stimulation of the hippocampal formation affect emotional reactivity, feeding behavior, motor activity, complex associative processes of conditioning, and even endocrine functions (Endroczi, Lissak, Bohus, & Kovacs, 1959; MacLean, 1957b; MacLean & Delgado, 1953).

Yet another difficulty arises from the fact that lesions and electrical stimulation both affect fibers that are passing through an area, as well as neurons and synapses (Grossman, 1970; MacLean, 1957a & b; Miller, 1965; Wilson, 1914).

A controversial literature concerns the behavioral and affective effects of either removal or stimulation of the amygdaloid complex and the closely adjacent neocortical and ventral hippocampus. Bilateral removal of temporal lobe structures has given conflicting results. Kluver and Bucy (1939) described a syndrome in monkeys consisting of psychic blindness (visual agnosia), strong oral tendencies, hypermetamorphosis (excessive tendency to attend and react to visual stimuli), hypersexuality, and hyperphagia. Emotional behavior was also considerably changed in the direction of increased tameness; there was a dramatic decrease in aggressive behavior and a loss of fear reactions.

Schreiner and Kling (1953) reported increased tameness in cats following lesions restricted to the amygdaloid complex and the overlying pyriform cortex. Walker, Thompson, and McQueen (1953) found that postoperative tameness and fearlessness in monkeys correlated fairly well with the amount of damage to the amygdaloid complex, but there were exceptions to this. Rosvold, Mirsky, and Pribram (1954) found similar behavioral changes to correlate with the extent of damage to the basolateral nuclei.

However, Bard and Mountcastle (1948) demonstrated that bilateral removal of the amygdala and part of the adjacent pyriform cortex in cats produced a striking and lasting lowering of the threshold of rage reactions. Their animals exhibited anger on the slightest provocation. Similar changes were reported by Spiegel, Miller, and Oppenheimer (1940).

There is no readily apparent explanation for these conflicting findings. Wood (1958), having produced "minute" bilateral lesions restricted to the central amygdaloid nucleus in the cat, described the consequent chronic

behavioral changes as "aggressive". Histological examination of animals rendered chronically aggressive indicated that the crucial lesion was that which led to secondary degeneration limited to the ventromedial nucleus of the hypothalamus, destruction of which produces permanent savagery. In an attempt to reconcile the conflicting behavioral changes reported in the literature, Wood suggested that because of "its inaccessible location in the cat, the amygdaloid nucleus, was very likely not removed in some ablation studies".

A careful study which included the observation of the normal behavior patterns in question, by Green, Clemente, and de Groot (1957), determined the anatomical substrate for two parts of the Kluver-Bucy syndrome in the cat. They ascribed male hypersexuality to bilateral lesions restricted to the pyriform cortex, and hyperphagia to lesions of the basal and lateral parts of the amygdala. However, these investigators also found that 16 of the 82 cats in their study suffered repeated convulsions and showed aggressive behavior during the interictal period. Histological studies showed that the lesions in all of these animals involved the ventral hippocampal formation to some degree.

The exact localization of emotional reactions elicited in the amygdaloid region by electrical stimulation has been much discussed. Ursin and Kaada (1960) found that stimulation of almost the same regions produced defense, as well as flight responses. MacLean and Delgado (1953) obtained responses of growling and sometimes hissing following electrical and cholinergic stimulation of the medial portion of the amygdala in cats. Magnus and

Lammers (1956) elicited growling from the medial nucleus as well as the central part of the basal nucleus in cats. These investigators also found that points yielding fear and restlessness were mainly localized in the periamygdaloid area and in the central, medial, and basomedial nuclei. Shealy and Peale (1957) reported rage reactions following stimulation of the central and medial amygdaloid areas in the same species of animal. They also obtained an "escape" reaction by stimulation of the basal and lateral components of the amygdala. The central area was sometimes involved too. By stimulation of the dorsal part of the basal nucleus and the adjacent portions of the central and medial nucleus in cats, Fernandez de Molina and Hunsperger (1957) described an "affective" reaction. Components in this reaction were growling, hissing, baring the teeth and laying back of the ears.

There is also some controversy as to whether it is possible to elicit by electrical brain stimulation pure behavioral syndromes. Hunsperger and his associates (1964, 1967) found that the flight and defense reactions can easily transfer from one point to the other during stimulation, and that both syndromes can be elicited from one point. He also described that combined stimulation of attack and flight areas intensified the attack response (Brown & Hunsperger, 1963).

Such contradictory results indicate that the identification of structures in the temporal lobes that are involved in either the Kluver-Bucy syndrome or Bard and Mountcastle's aggressive cats is still needed. It appears then that experimentation using ablation or stimulation techniques,

particularly when these devices are used to approach complex problems of emotional behavior and their anatomical analysis, is fraught with the influence of numerous variables, not the least important of which is stimulus intensity.

Because of the different threshold of nerve fibers and neurons to electrical and cholinergic stimulation, chemical stimulation employing putative neurotransmitters should produce effects which are more specific to given functional systems than effects produced by electrical stimulation, and may act on synapses and soma without affecting axons or fibers of passage (Lorente de No, 1944, Stevens, Kim, & MacLean, 1961).

Biochemical investigations have identified a number of potential synaptic transmitter substances that are distributed nonrandomly in the central nervous system (Feldberg & Vogt, 1948; Forster, 1945; Fuxe & Gunne, 1964; Vogt, 1954). Specifically, choline-acetylase and cholin-esterase are distributed in areas which have been shown to be extremely sensitive to cholinergic agents and that are not excited by glucose, sodium, epinephrine, norepinephrine, serotonin, dopamine, etc. (Feldberg & Vogt, 1948; MacLean, 1957a; MacLean & Delgado, 1953). When administered systemically or centrally, these agents produce selective behavioral effects that suggest a specific action on functionally defined neural systems. These observations indicate that synaptic transmission may be chemically "coded" in areas of extensive anatomic overlap in such a way that system A may respond only to transmitter X, while immediately adjacent synapses of system B respond only to transmitter Y. It should then be possible to excite or inhibit specific functions selectively by local applications of

the transmitter or substances which potentiate, mimic, or inhibit its action.

Recent findings support the contention that chemical stimulation procedures may be significantly more selective than conventional electrical stimulation or ablation methods. A number of investigators have used the chemical stimulation technique to delineate particular behavioral patterns which appear to be chemically coded (Grossman, 1960, 1962; Hernandez-Peon, Chavez-Ibarra, Morgana, & Timo-Laria, 1963; MacLean & Delgado, 1953; Miller, 1965; Myers & Sharpe, 1968; Myers & Veale, 1970).

More specifically, microinjections of chemicals which initiate, facilitate, or inhibit synaptic transmission have been used extensively to produce temporary changes in the activity of a discrete brain area, namely the basal amygdaloid complex. As previously mentioned, it has been shown by ablation and electrical stimulation techniques, and chemical stimulation studies as well (Delgado, 1964; Kaada, Anderson, & Jansen, 1954), that this specific region of the limbic system is concerned with a particular emotional response - aggressiveness or savageness.

In general, the chemically induced changes are reversible, and the organism appears electrophysiologically and behaviorally normal within a few hours after the drug application. A notable exception, however, has been reported after chemical stimulation of the basal amygdaloid complex bilaterally with crystals of the cholinergic substance, carbamylcholine (Grossman, 1963). Carbamylcholine (carbachol) is a parasympathomimetic drug which is not hydrolized by cholinesterase and, therefore, produces a

prolonged excitatory effect on postsynaptic membranes, and generally mimics the effects of acetylcholine at peripheral neuromuscular junctions (Koelle, 1970). Intracerebral injections of minute doses (2.5-5.0 µg) of this chemical into the basal medial amygdaloid nucleus of the cat induced epileptiform seizure activity which gradually spread to other regions of the brain and eventually involved all areas from which recordings were obtained (sensory and motor cortices, frontal lobes, thalamus, hippocampus, septal area, hypothalamus, and midbrain reticular formation). This generalized electroencephalographic seizure was accompanied by violent convulsions. After several hours of uninterrupted seizure activity, the electroencephalographic records began to show gradually lengthening periods of more normal, low amplitude activity, accompanied by a gradual lessening of the convulsions. In the course of the following week, the animals recovered to the point where they could walk well, maintain adequate nutritional and fluid levels by voluntary intake, and resume normal sleep-activity cycles. However, all animals remained extraordinarily vicious and attacked man, as well as other animals, without provocation or apparent regard for their own safety.

Some of these cats were observed for as long as five months. They remained vicious and uncooperative throughout this period in spite of repeated attempts to make them more docile by handling and petting. Abnormal electroencephalographic activity consisting of low voltage fast waves and random spikes also persisted in all areas of the brain. Histological examination of the tissue surrounding the cannula implant failed to show any evidence of abnormal tissue reaction at the tip of the cannula, suggesting

to the author that the single application of carbachol may have produced permanent functional changes.

In a subsequent study of the one-way avoidance performance of a group of rats treated with a single bilateral injection of crystalline carbachol into the amygdala, Belluzzi and Grossman (1969), concluded that the effects of carbachol on behavior were primarily on inhibitory systems, since the administration of the cholinergic blocking agent, scopolamine, restored the animals' ability to inhibit a response. On the basis of further evidence that behavioral changes lasting for several days have been seen following the electrical stimulation of the amygdala (Alonso de Florida & Delgado, 1958); and that a permanent reduction in amygdaloid seizure threshold can be produced by repeated low current electrical stimulation of that region on a restricted time schedule (Goddard, 1967), Belluzzi and Grossman (1969) concluded that the cholinergic stimulation of the amygdaloid region produced long-lasting functional changes indicative of a lowered threshold for discharge of amygdaloid neurons following chemically induced activation. Grossman interpreted this as evidence of enduring "facilitatory" effects, which he likened to "post-tetanic potentiation," and suggested that the amygdaloid neurons were predisposed to sustained depolarization following specific stimulation schedules or modes.

Alternatively, it is equally likely that the profound emotional and electroencephalographic changes are caused by either the direct destruction by carbachol crystals of an amygdalar region which inhibits aggressiveness, or the indirect destruction of the injected area and associated projection

systems by the violent seizure discharges (Crowell, Wyss, Fankhauser, & Akert, 1968) which usually follow such injections; as by a chemically-induced depolarized limbic subsystem which potentiates the abnormal affective responses of savageness and aggressiveness. Yet, as mentioned above, no lesions were reported by Grossman. Neither were tests for possible alteration of local excitability of the injected site or projection areas undertaken to test his hypothesis of augmented excitability as the major etiological factor underlying the savage behavior.

The major purpose of the present investigation was to determine whether the enduring behavioral effects of carbachol stimulation of the basal amygdaloid complex as originally described by Grossman (1963) were due to (1) chronic hyperexcitability (defined as a decrease in after-discharge threshold or (2) a lesion (defined as an increase in after-discharge threshold) at the site of chemical stimulation. Such a decision should also allow a more precise conclusion concerning the earlier conflicting evidence that lesions of the amygdala either release or abolish savage behavior in the cat.

To accomplish this purpose chronic savage cats prepared with insulated cannulas implanted in the basolateral or basomedial amygdala and recording electrodes in the anterior or ventromedial nucleus of the hypothalamus, the hippocampus, and neocortex, according to the method of Grossman, were to be subjected to electrical stimulation and lesions at the site of the original carbachol instillation, to determine the effect of each on the chronic rage state.

The use of the after-discharge threshold to determine the pre- and postcarbachol electrical threshold also permitted the investigation of a second question, i.e., comparison of the behavioral and electroencephalographic effects of cholinergic and electrical stimulation of identical sites in the brain of the chronically prepared freely moving animal. Following the determination of the electrical after-discharge threshold of the amygdala at the tip of the insulated cannula, 15-25 µg of crystalline carbachol were injected through the same cannula into the identical brain locus. Recovery from the acute effects of the drug was allowed before further amygdalar after-discharge threshold levels were determined in order to compare pre- and postinjection thresholds.

The simultaneous behavioral and electroencephalographic observations reported here attempt to answer these two questions, namely: (1) Whether the enduring behavioral effects of carbachol stimulation of the basal amygdala were due to chronic hyperexcitability or hypoexcitability in areas anatomically or physiologically associated with the site of chemical stimulation; and (2) The comparison of behavioral and electoencephalographic reactions following stimulation of identical brain areas with electrical pulses and a cholinergic agent.

METHODS

Subjects

The experiments were performed on 23 adult cats of either sex weighing 2.6-3.5 kilograms. These animals were purchased from the University of Oregon Medical School Farm and were all born and raised in this facility, spending their entire lives in enclosed runways. In the first series of experiments, designed to replicate the Grossman postcarbachol enraged cats, 7 cats had amygdala cannulas implanted in the basolateral amygdala and 3 cats had amygdalar cannulas placed basomedially. In a second series of experiments designed to compare, in addition, the issue of electrical versus chemical stimulation, 9 experimental and 2 control cats were implanted with amygdala cannulas basomedially, while 2 additional experimental animals received basolateral amygdalar cannulas.

Materials

Amygdala cannulas were prepared from 21-gauge (0.032 in. OD) stainless steel tubing (Tubesales, Los Angeles, California). The outer surface of these cannulas was insulated with either Formvar (No. 35, extra heavy, Phelps Dodge Copper Products, Phelps, Indiana) or Epoxylite (No. 6001-M, Epoxylite Corporation, El Monte, California),except for the cross section of their tip, and were fitted with stylets which protruded 0.5 mm beyond the tip. Recording electrodes were either single or double (twisted) lengths of stainless steel wire (0.01 in.OD) insulated with teflon except for a scraped 0.5 mm tip (No. 316, Driver-Harris, Harrison, New Jersey).

Single stainless steel teflon-coated wires were soldered to the amygdalar cannulas above their emergence from the skull to permit their use as stimulating or recording electrodes at the site of electrical and chemical stimulation. These amygdalar cannulas were easily converted to bipolar concentric stimulating electrodes by introducing another single teflon-coated stainless steel wire, insulated to within 0.5 mm of the tip and protruding by 0.5 mm from the base of the cannula, which acted as the inner cathodal pole of the stimulating electrode. All electrodes had Amphenol male contacts (No. 220-P01-100, Amphenol Corporation, Chicago, Illinois) which were fitted into a female Amphenol plastic plug (No. 221-9160). The cannulas, electrodes, and plastic plug were fixed to the skull with Unitek cold cure dental acrylic (Unitek, Monrovia, California).

During experimental sessions, the animal was housed in a glass-fronted recording and observation box (12 x 24 x 12 in.) with an opening for the electrical cable at the top. The cat was attached to a multilead recording cable by a male Amphenol plastic plug (No. 221-1260) which was fitted in turn with Amphenol female electrode contacts (No. 220-S01-100). Between recording sessions, the animals were housed in plastic-lined individual cages in the animal care quarters and maintained on commercial cat food and water.

Both an 8-channel Grass Electroencephalograph Modell III-D Console and a 4-channel Grass Model 7 Polygraph were used to record the cerebral electrical activity. The electrophysiological data of 3 cats was simultaneously stored on magnetic tape by a 7-channel FM-Hewlett-Packard tape

recorder with impedance-matched Grass P-511 series ac preamplifiers. The tape recorded data was monitored at the time of acquisition by a 4-channel Tektronix 564 storage oscilloscope.

Electrical stimulation was performed with two Grass S-5 stimulators connected through Grass Stimulus isolation units and impedance-matched circuits. Current intensity was monitored on a dual beam Tektronix 502 oscilloscope by observing the voltage drop across a 100-ohm series resistor. The signal for the electrical stimulus was provided by a 1-V square wave which was recorded directly from the Grass stimulators through two Tektronix type 161 Pulse Generators and one Tektronix type 162 Waveform Generator operated from a Tektronix type 160A Power Supply onto the seventh channel of the tape recorder.

Injected carbamylcholine (carbachol) was either in liquid (Doryl U.S.P. Merck) or crystalline (Carcholin U.S.P. Merck) form. Liquid injections were made with Glenco microsyringes, while crystalline deposits were made with Becton Dickinson Yale Luer-Lok stainless steel 26-gauge (0.018 in. OD) hypodermic needles cut to the correct length. Crystalline carbachol was used most frequently, both to replicate the chronic results obtained by Grossman, and because it has been shown that liquid injections tend to run up along the needle tract and, therefore, are more widely dispersed (MacLean, 1957a).

Analysis of the evoked potential data was carried out by a Nuclear Data Enhancetron 1024 with the write-out provided by a Tektronix 503 oscilloscope and a Tektronix C-12 polaroid oscilloscope camera. Amplification

of the amygdalar activity which triggered the analysis of the hypothalamic potentials was accomplished with an Analog Devices 350-A amplifier operated by an Analog Devices Model 902 dc Power Supply in series with a Tektronix type 161 Pulse Generator and a type 160A Power Supply. The Analog Devices amplifier plus the Tektronix Pulse Generator converted the 1-V tape recorded data to approximately 8 V which was sufficient to trigger the Enhancetron.

Procedure

The following procedure was carried out in each cat. Food was withheld for 24 hours prior to surgery. The animal was premedicated with a small dose of atropine sulfate (0.033 mg/Kg) intramuscularly. The cat was then anesthetized with an intraperitoneal injection of 35 mg/Kg of sodium pentobarbital (Nembutal) and secured in a Kopf stereotaxic apparatus.

The scalp was cut along the midline, retracted back, and scraped clean of its periosteum. Three small holes were drilled part way through the skull: one hole 5 mm anterior to the coronal suture and 1 mm lateral to the saggital suture, over the frontal sinus, and two holes 4 mm posterior to the junction of the saggital sutures and 12 mm lateral to the midline, over the occipital cortex. Stainless steel screws were then inserted into these holes to serve as anchors for the electrode plug. A thin layer of cold cure dental cement was spread between the founcation screws to secure them properly to the skull, as well as to provide a cement base for attachment of the electrodes.

The cannulas and recording electrodes were stereotaxically implanted under standard sterile technique in the limbic system and cortex according to the coordinates of Snider and Niemer (1961). Table 1 indicates the

anatomical location of implanted cannulas, as well as other pertinent information pertaining to the chemical stimulation procedure.

For both chemical and electrical stimulation, the two 21-gauge insulated cannulas were bilaterally placed in either the basal medial (A- +12.5; L- ±9.0; V- -6.0) or basolateral (A- +12.5; L- ± 12.0; V- -5.0) portion of the amygdaloid complex. These cannulas were used for the subsequent introduction of the 26-gauge stainless steel needles of identical length containing measured amounts of crystalline carbachol, and for the introudction of insulated stylets with light-weight lead-off wires, used with the insulated cannula as the outer pole for bipolar electrical stimulation and recording.

In addition to the insulated cannulas, bipolar recording electrodes were implanted bilaterally into the dorsal hippocampus (A- +2.0; L- ±9.0; V- +7.5), the anterior hypothalamus (A- +13.5; L- ±1.3; V- +2.3) in the first series of experiments, the ventromedial hypothalamus (A- +10.0; L- ±1.9; V- -6.0) in the later group of experiments, and in some animals the septum (A- +15.0; L- ±2.0; V- +3.5). Monopolar surface electrodes were bilaterally placed over motor (A- +22.0; L- ±10.0) and occipital (A- -4.0; L- ±3.0) cortices. A length of stainless steel suture embedded in the temporal muscle served as the indifferent electrode. Cannulas and electrodes were fixed to the skull by acrylic cold cure dental cement. The male Amphenol metal contacts of the electrodes were fitted into a female Amphenol plastic plug and fixed solidly to the skull with the dental acrylic according to a technique developed by Milstein (1969). Correctly prepared cats handled in this way were in lively, healthy condition and were used over a period of months for recording and stimulating experiments.

On the seventh postoperative day, the animal's recording electrodes were checked for broken or shorted circuits. At this time, a recording montage was determined which was used to monitor all subsequent electrical and chemical procedures in that animal. Over brief sessions on subsequent days, the cat was habituated to the recording apparatus where he was able to move about in a relatively free manner, although attached to the skull plug to the light-weight recording cable.

Previous to any experimental manipulations, an estimate of the base line emotional and electroencephalographic behavior of the experimental and control animals was obtained by administering several short behavioral tests. A specially prepared behavioral rating scale examining several facets of feline behavior was utilized (Appendix 1). The subject made one of five possible responses to each behavioral test. An animal showed: (1) a maintained positive interest towards the stimulus; (2) an immediate interest in the stimulus which was not maintained; (3) a maintained escape response away from the stimulus; (4) escape behavior away from the stimulus which was not maintained; or (5) no interest in the presented stimulus.

A sensory examination assessed the animal's behavioral and electroencephalographic responses to both noxious and pleasant stimuli. Visual perception was tested by reactions to a bright light versus a dim one; an ether soaked versus a tuna fish soaked gauze assessed olfaction, a pin prick and chucking under the chin determined tactile sensation; and a loud noise versus affectionate verbalizations tested audition. Also, special attention was paid to their reaction to the approach of the observer;

to opening the cage door; to handling, stroking, and petting; to tail and paw pinching; to other animals; and to the introduction of a catnip mouse and a gloved hand or stick into its cage. Additional observations were made of motor activity, vocalization, and response to food. These behavioral tests were repeated several times in order to establish both base line behavioral and electroencephalographic responses before proceeding to the electrical and chemical stimulation phases of the study.

On the eighth day following surgery, basal amygdala regions of experimental and control animals in the second series of experiments were electrically stimulated to the point of after discharge, and the latency and shape of the averaged evoked response from monitored ventromedial hypothalamic regions was determined, as well as the behavioral effects of the procedure.

Electrical stimulation was provided by biphasic rectangular pulses of 1.0 millisecond duration applied for 10 seconds. Two frequency settings were used: 3 cps pulses applied for 10 seconds served for subsequent analysis of averaged evoked potentials recorded from the ventromedial nucleus of the hypothalamus, followed by 10 cps pulses administered for 10 seconds in order to elicit the after discharge. Electrical stimulation began at 0.2 mA and was raised in 0.2 mA steps for each successive train at 2-minute intervals until a definite after discharge occurred.

Several days or weeks following the electrical stimulation procedures, the identical locus of the amygdala was chemically stimulated in experimental animals through the same electrode and similar measurements were

made. Ten microliters or 10-25 micrograms of carbachol were bilaterally injected into the basal amygdaloid region. Crystalline carbachol was spread evenly on a watchglass and either 3 or 5 taps of carbachol were tamped into the tip of the injecting needle. Close approximation to these minute amounts was best obtained by the tamping method devised by Baxter (1967, 1968). Based on the microbalance weighing of pieces of stylet packed with various numbers of taps of carbachol, he found that one tap $\simeq 10\mu g$ of carbachol, while five taps $\simeq 25\mu g$. In order that the excitant agent did not come into contact with fluid or tissue at the base of the cannula, a crystal of glucose was tamped over the carbachol at the tip of the injecting needle prior to injection in most animals. After the needle was introduced to the base of the guiding cannula, the substance was ejected by the stylet. Following the chemical injection, the animal was closely observed and continuous electroencephalographic recording was undertaken throughout subsequent seizures, or for 2-3 hours if no behavioral change ensued. When uninterrupted seizure activity followed chemical implantation and appeared life-threatening, barbiturates were cautiously administered to suppress the epileptic activity. Intramuscular injections of luminal (6.0 mg/Kg), as well as cortisol (0.06 mg/Kg), were administered every 4 hours during the first 24 hours in 7 cats; thereafter, medication was gradually withdrawn. Other supportive care at this time included the administration of fluids, nutrients and continuous nursing care.

Animals surviving the carbachol implantation were subsequently tested for after-discharge threshold level at intervals up to 7 weeks following

the chemical stimulation in order to compare the preinjection and post-injection thresholds. Following both the electrical and chemical stimulation procedures, the behavioral check list was repeated in order that the pre- and postmanipulatory behavior and electroencephalographic status of an animal could also be compared.

Cinematographs documenting specific behavioral alterations following the experimental procedures were taken. One of the greatest problems in the study of behavior is that most spontaneous and evoked reactions defy instrumental analysis. By use of photography and accurate detailed direct observations, complex behavioral patterns such as threat, attack, defense, and fear were documented.

Following the final electrical stimulation procedure, electrode placements were histologically confirmed in 15 experimental and 2 control animals. These cats with either sacrificed with an overdose of intraperitoneal Nembutal and their brains directly removed from the skull after death and stored in 10% formalin, or were anesthetized with an intraperitoneal dose of Nembutal and perfused via the ascending aorta with normal saline followed by a solution of 10% neutral formalin for gross and histological examination. The needle tracts for subcortical electrodes were located and whole brain slices containing the cannula tracts were removed for fixation at appropriate levels. Six and 15 μ sections were cut and stained with Hematoxylin and Eosin and Luxol-Fast-Blud-periodic-Acid-Schiff stains according to the standard procedures followed by the Department of Neuropathology at the University of Oregon Medical School. Sections were examined for accuracy of electrode placement, local cellular destruction, and fiber tract degeneration.

Averaged evoked hypothalamic activity time locked to the electrical pulse delivered to the amygdala was compared to the hypothalamic activity immediately following the carbachol-induced amygdala spikes (prior to the obvious generalization of this spike activity to the hypothalamus), utilizing the chemically-induced train of amygdala spikes as the stimulus for selecting coincident 250 millisecond samples of the ipsilateral hypothalamic activity. For comparison of the averaged evoked response, 30 250-millisecond segment samples of hypothalamic activity were averaged at each stimulus intensity prior to the occurrence of an after discharge. For the chemical activation procedure, 30 250-millisecond segments of hypothalamic electrical activity were averaged from the carbachol-induced single spikes that preceded visible onset of sustained spike discharge in the hypothalamus at similar time intervals.

RESULTS

In presenting the specific results of the experiments performed in this study, the following format will be used:

I. Postoperative Behavior
    A. Postoperative Recovery Period
    B. Postoperative Personality
II. Chemical Stimulation Behavior
    A. Acute Ictal Behavior
        1. General Description
        2. Anger-Attack Behavior
        3. Fear-Defense Behavior
        4. Fear-Escape Behavior
        5. Unprovoked Rage Behavior
        6. Focal Discharge Without Behavior or Generalized Seizures
        7. Electroencephalographic Responses Without Behavior
        8. No Electrical, Clinical or Behavioral Change
    B. Chronic Postictal Behavior
        1. Overirritability
        2. Overdocility
        3. Overfearfulness
        4. No Behavioral Change
III. Electrical Stimulation
    A. After-Discharge Threshold Level
    B. Averaged Evoked Potentials

IV. Histology

I. Postoperative Behavior

A. Postoperative Recovery Period

Recovery from surgery and anesthesia was usually uneventful, as each animal sat up and walked about with no apparent discomfort within a few days. Cats who remained comatose for more than 24 hours postoperatively were given supportive nursing care in the form of subcutaneously administered fluids and nutrients.

B. Postoperative Personality

In general, the behavioral patterns of the cats were two fairly easily recognized qualitative types which, for descriptive purposes, will be referred to as playful and withdrawn. The playful friendly cat spontaneously engaged in play with the observer, as well as other cats. It was active in its cage, walked boldly about the animal care room, rubbed head and body against the cages, and purred. When approached, it came forward, rubbed against the hand, purred, and playfully pawed. It could be picked up with no attempt to escape and purred all the while. Such cats jumped out of their cages voluntarily when the door was opened, and once on the floor, some rolled over on their backs. Allowed unrestricted movement on the floor, they wandered about investigating freely, and frequently came up to the observer to rub against him. Pinching their tails or paws elicited escape activity and meowing, but with more intense stimulation they would strike out with their claws. On discontinuance of the pinching, the cats could be easily handled, and they quickly regained their friendly attitude.

Before habituation to the observation and recording box, these cats frequently attempted to disconnect the recording cable by rolling, twisting, turning over on their backs, and at times even directly attacking it with their teeth and claws. Characteristically, these cats restlessly wandered about the box meowing in a protesting manner and sometimes attempted to climb out of the apparatus, through the opening for the recording cable.

The behavioral characteristics of the withdrawn quiet cat were, in general, the opposite of those of the playful cat. These animals sat far back in their cages and looked about with a general attitude of attention or alarm. When approached, this cat characteristically withdrew from the observer, as well as other cats. Upon opening the cage door, the cat did not voluntarily come out of the cage but had to be pulled out. The quiet cat could not be picked up without some difficulty. It clung to the observer with its claws and attempted to escape whenever possible. Allowed to roam freely about the animal room, the cat frequently hid under the cages and would not come out when called. Intense noxious stimulation such as pinching their tails or paws elicited meowing and escape activity, but never a threat or striking posture. In general, then, the behavior of the quiet, withdrawn cat was suggestive of apprehension or fear.

A clear distinction between the two cat personality types was also evident in each animal's reaction to the sensory and social behavioral test battery. In general, the final response of all the animals to the sensory stimuli was similar in that noxious stimuli were usually avoided while pleasant ones elicited either indifference or approach behavior. However, the initial reaction to each stimulus situation was different in that the

playful cat usually inspected and sniffed at everything unfamiliar while the quiet cat withdrew from strange stimuli in a frightened fashion. Also, the playful cat, whether male or female, usually showed a slight to strong lordosis in response to back stroking, while the withdrawn animal would always back away in an attempt to escape from the situation.

In general, the acute ictal and chronic postictal behavioral manifestations of the animals were not predictable from the pre-experimental personality of the cat.

II. Chemical Stimulation Behavior

A. Acute Ictal Behavior

1. General Description. Following the deposition of carbachol bilaterally into the basal amygdaloid complex, behavioral reactions with an emotional coloring were elicited from this structure. In general, most of the responses described here were automatisms seemingly related to the situations of fear, defense, and aggression.

The first observable reaction was an alerting response which consisted of an abrupt cessation of spontaneous ongoing behavioral patterns such as drowsing, grooming, walking, and purring. A few minutes later, following the sudden occurrence of bursts of low amplitude amygdala sharp activity, the animal began some investigative activity characterized by restless searching and sniffing of the floor and walls of the observation box. Soon after the onset of the regular amygdala activity, the eyes of the animal darted to and fro with the head following the searching eye movements (Figure 1b).

As the intensity of the amygdala activity increased, somatomotor (sniffing, licking, chewing, contraction of the facial muscles), as well as autonomic reactions (changes in respiration and heart rate, salivation, pupillary dilatation), occurred. During this stage of development, the abnormal activity in the electroencephalogram consisted of a rhythmic discharge of high amplitude spikes varying in frequency from 8-14 cps interrupted by periods of fast activity (Figure 1c).

During the early stages of the carbachol action, the periods of spontaneous regular sharp activity were short and sometimes lasted for a few seconds, interrupted by periods of silence; later they extended to more than 5 minutes in duration.

Electrical seizure discharges typically began with short bursts of sharp activity from either the right or left amygdala within 5-20 minutes following the bilateral carbachol implantation. Often the beginning was characterized by regular rhythmic 60-70 μv theta activity from the focal amygdala electrode. During the first 15 minutes following the injection of the cholinergic drug, the bursts of sharp activity were restricted to the subcortical leads from a single amygdala and after 5-10 minutes from the ipsilateral hypothalamus, without an evident change in the cortical electroencephalographic record. If further propagation ensued, the electrical discharge became evident in other amygdalar, hypothalamic, and hippocampal subcortical regions, and finally in the cortex. It was at this time that spontaneous alterations in affective behavior were apparent. The general pattern of activation of cerebral structures then was as follows.

The electrical discharge began in one amygdala and spread to the ipsilateral hypothalamus within 15 minutes of onset. A few minutes after the hypothalamic involvement was evident, ipsilateral septal and hippocampal and contralateral amygdalar structures were included in the discharge. A few minutes later, the simultaneous discharge of spike and slow wave complexes from amygdalas bilaterally signalled the onset of a rhythmic, synchronous electroencephalographic discharge involving all monitored cortical and subcortical regions; consisting of high amplitude (200-300 μv), 3-6 cps spike and slow wave discharges. Sometimes a fully developed electrical discharge stopped abruptly and was replaced by low voltage fast activity from all regions. During the next 30-40 seconds, no abnormal spontaneous rhythm occurred. When again apparent, discharges were initially small and restricted to the amygdalas and hypothalami bilaterally; but quickly increased in amplitude as they appeared in the other subcortical and cortical regions. Although epileptiform activity usually demonstrated a delayed onset from several minutes to 20 minutes, accompanied by a progressive build up, occasionally the effect appeared within 10 seconds of injection (BM-2879, BM-4016). Focal motor seizures occurred in 13 cats, and bilateral tonic-clonic seizures involving the entire body ensued for a few hours to 48 hours in 9 of these cases. Convulsions were often preceded by salivation and a state in which the cat gave the impression of being either confused or distracted by hallucinatory stimuli. In this condition, the cat either moved about restlessly or remained intently still as it hypervigilantly watched the wall of the observation box.

Grand mal attacks typically began with twitching of the ears and whiskers, and clonic movements of the head developing into clonic movements of the whole body. Accompanying these clinical epileptiform signs was an increase in the amplitude and frequency of the background activity of one amygdala, which spread to the ipsilateral hypothalamus and contralateral amygdala as the generalized seizure developed (Figures 2a-j).

Frequently, the cat then fell, with head and neck flexed or rolled over, jerking in rapid clonic movements. Typically, this clonic stage varied from 20.0-85.0 seconds and was followed by a few minutes of postictal depression in which the animal lay in a stupor on the grid floor of the observation box, with greatly accelerated respiration and heart rate, and maximally dilated pupils, apparently exhausted and dazed. At this point, the introduction of a strong noxious stimulus such as a pin prick failed to elicit any avoidance or other response.

If no steps were taken, a second attack of major convulsions developed after a short time. Four animals died during the postictal exhaustion state (BL-2607, BL-2654, BM-2683, BL-2716). Even with the administration of anticonvulsant medications in subsequent cats, two still succumbed within a few hours of their first grand mal convulsion (BM-2878, BL-3044).

Between seizures, other cats (BM-2736, BM-2778, BM-4016) maintained bizarre catatonic stances or developed hypervigilant behavior which lasted as long as 20 minutes. For example, Cat BM-4016 remained standing in an awkward upright position with front paws widely extended, the back slightly arched, tail in the air, staring into the corner of the box, for as long

as 15-20 minutes, during which the electroencephalograph showed high amplitude 150-200 μv, 1-2 cps spike and slow wave potentials from all regions of the head (Figure 3b).

Anticonvulsant medications or spent neurons commonly brought the generalized epileptic activity under control within 24 hours of the chemical injection. At this time, most cats had resumed spontaneous eating and drinking, but were still weak and lethargic. The electroencephalographic record had usually returned to its preinjection state or showed episodic runs of slowing or typically demonstrated slow 1-2 cps intermittent spike activity. Surface, hippocampal and hypothalamic sites had returned to their previous state of low voltage fast activity (Figure 3d). However, during the first 48-72 hours postictus, moderately strong affective stimuli now typically elicited rhythmic bursts of high voltage spike activity of varying frequency associated with pathological affective behavioral responses. Figures 3e and 3f show bursts of these spike potentials from noxious olfactory stimulation with ether, which the cat actively sought and rolled in (Figure 4), and calling "kitty kitty" to which it rolled over on its back and purred. Within 7 days, the electroencephalographic record had lost these spikes in response to affective stimuli, and either returned to normal or displayed low voltage slow activity from amygdalar subcortical sites (Figure 5g).

To summarize the clinical and electroencephalographic data, 3 cats (BM-2683, BM-2878, BL-3044) developed immediate generalized grand mal convulsions, and died within 2 hours of bilateral injection of crystalline carbachol into the basal amygdaloid complex. Three animals (BL-2607,

BL-2654, BL-2617) displayed subsequent grand mal seizures which developed from original focal motor attacks within 12 hours of chemical implantation and died in status epilepticus within 2-4 days of onset.

Three cats (BM-2778, BM-2902, BM-4016) survived generalized seizures which had progressed from initial focal motor epileptiform activity; while 4 animals (BL-2683, BL-2728, BM-2736, BM-2850) recovered from focal motor seizures that never progressed to generalized convulsions. Weak to well developed signs of localized electrical epileptiform activity that disappeared within a few to 24 hours without overt clinical evidence of a seizure discharge was displayed by 6 cats (BM-2690, BL-2723, BM-2729, BM-3014, BM-4014, BM-4052). Finally, 3 animals failed to demonstrate either a clinical or electrical response to the carbachol implantation (BL-2716, BL-2725, BM-4015).

2. Anger-Attack Behavior. Ictal anger-attack behavior was shown by 3 cats (BL-2607, BM-2729, BM-2902). Typical aggressive attack reactions included mydriasis, strong retraction of the ears, lowering of the head, arching of the back, protrusion of the claws, and extension of the legs. Low growling, hissing and striking and lashing of the tail were accompanied by signs of sympathetic discharge such as pupillary dilatation and piloerection. When the cat was approached, it bared its fangs, retracted its ears, arched its back, and snarled and hissed with increasing violence (Figure 6b). With stronger approach, any slight movement by the observer provoked a violent, well-directed attack. When a stick or gloved hand was offered, the cat raised a forepaw, ready to strike, accompanied by vigorous

hissing. Some animals even attacked these objects fiercely with their teeth and claws. This angry behavior in which the cat would growl, hiss, and strike out if interfered with lasted as long as 20 minutes.

A specific form of short-lasting anger-attack behavior was observed in 2 cats (BL-2683, BL-2728) that received injections of liquid carbachol. Within a few minutes of applying 10 γl of acqueous carbachol bilaterally to the basolateral portion of the amygdala, high voltage rhythmic 8-12 cps spike activity was recorded from the sites of chemical stimulation, as well as hypothalamic, hippocampal and surface regions. At this time, the animal was observed to hiss and growl intermittently. If directly approached, it would bare its fangs and claws. For a few moments later, a similar approach would result in well-directed attack behavior. About 10 minutes after the injection, the abnormal electroencephalographic activity had subsided and the animal again appeared friendly in that there was nothing unusual about his behavior.

3. Fear-Defense Behavior. In these cats (BL-2654, BM-2778) the first recognizable reactions to the chemical implant consisted of mydriasis, looking about, and a general attitude of alarm. As the intensity of the amygdala discharge increased and spread to other hypothalamic, hippocampal, and septal limbic structures, more striking responses were observed. Soon after the onset of amygdala spikes, the pupils dilated and the palpebral fissure widened. Snarling, hissing, slight retraction of the ears, and looking about appeared in rapid succession. This aggressive pattern was not always distinguishable from the aggressive pattern above; except that

characteristically, these cats withdrew from rather than attacked the experimenter. A gloved hand or stick thrust into the cage was avoided as the cat looked about as if in search of an escape, even when the animal was simultaneously growling, hissing, and pawing with extended claws; whereas in aggressive-attack reactions, these objects were attacked with teeth and claws.

4. Fear-Escape Behavior. This acute behavioral reaction to the chemical stimulation was shown by 2 cats (BM-2690, BL-2723). Similar to the ictal fear-defense animals, one of the first observable reactions of these cats consisted of quick searching movements of the eyes and head as the animal looked about in a state of apprehension or alarm. This reaction was associated with the onset of regular bursts of 8 cps moderate amplitude 100-150 μv spike activity from the amygdala unilaterally (Figure 1b). As the intensity of the amygdala discharge increased, the cat behaved in a frightened manner by looking wildly about as if trying to seek an escape (Figure 1c).

A few minutes later, the amygdala spikes began to discharge at a continuous steady rate of 12 cps (Figure 1d), and the ipsilateral hypothalamus and contralateral amygdala and hypothalamus demonstrated an attenuated spike discharge of a slower frequency (Figure 1e). The cat behaved in a frightened fashion in that it maintained a hypervigilance as if watching something terrifying. At this time, no behavior could be provoked by introducing extraneous stimulation, such as a gloved hand or stick. In general, the cat behaved as if it were caught up in its own imaginary world of terror (Figures 7a-d).

5. Unprovoked Rage Behavior. In order to demonstrate this specific result, as seen in 2 animals (BM-2778, BL-3044), all electroencephalographic illustrations were taken from the protocols of cat BL-3044, which was representative of this result. Figure 2a shows a typical record during the control period immediately preceding the carbachol stimulation. The cortical record frequently displays the conspicuous rhythmic spindles associated with quietude as shown in channels 3 and 6, while relatively fast, low level activity is usually recorded from subcortical sites, for example, channels 1, 2, 4, and 5.

Approximately 5 minutes after the second bilateral injection of crystalline carbachol, low voltage fast activity replaced the spindles in the cortical record, and was immediately followed by a slow build up of a rhythmic spike discharge from the left amygdala. Appearance of the cortical "activation" pattern is typical of the initial stage of seizure discharge from limbic structures in experimental preparations and also of a human seizure discharge originating in this region (Gloor, 1957; Jasper, Pertuiset, & Flanigin, 1951).

A constant rhythmic discharge of amygdalar sharp activity (50-80 μv) began at 12 cps and increased in frequency to 15-16 cps within 10 seconds (Figure 2b). This first major discharge lasted 13 seconds when the left amygdala discharge rate abruptly changed to a relatively steady rate of 15 cps in bursts of decreasing duration (from 5.0-1.0 sec), separated by a 500-millisecond interictal period (Figure 2c). At this time, spikes of similar frequency appeared from both the left hypothalamus and septum. During this time, the cat ceased all motor activity and sat very quietly while staring intently at a wall of the observation box.

Suddenly, the regular left-sided amygdala sharp waves became 6 cps spike and slow wave potentials which rapidly decreased in rate to a steady rhythmic 3 cps, 100-120 μv discharge. At this time, the right amygdala and the left hypothalamus and septum all showed the regular 3 cps abnormal activity with a suggestion of a similar pattern of activity in the right hypothalamus and septum, and the motor and occipital cortices (Figure 2d). The cat's pupils were by now dilated and he salivated profusely and masticated the saliva, chewing and swallowing repeatedly. The record gradually changed to 1 and 3 cps spike and slow wave bursts with 1-second interictal periods in which the left amygdalar-hypothalamic activity was suppressed and replaced by low voltage flat potentials, while all other cortical and subcortical placements showed low voltage fast activity (Figure 2e). The animal continued to salivate, then suddenly jumped to the right at the same time that the left eye began to twitch. Brief, relatively strong stimuli such as calling, loud clapping or yelling, did not interfere with these bursts or distract the cat from his compulsive behavior.

The left-sided hypothalamic-amygdala activity slowly changed to larger amplitude 150-200 μv steady 1 cps spike and slow wave potentials (Figure 2f), associated with a synchronous blinking of the eyes, while the animal slowly backed away from the front of the recording box as though escaping from something terrifying. As the left hypothalamic activity slowed to a 1 cps slow wave, some regular 16 cps rhythmic sharp waves became superimposed on the slow wave following the 1 cps spike in the right amygdala (Figure 2g). This activity was associated with left-sided facial twitchings and a rigid

motionless, vigilant stance as the animal stood with its tail in the air staring at the corner of the box. Touching the cat at this point produced a rapid upward deflection of the tail but no real contact with the observer and no conspicuous change in the electrical record.

Again, the electroencephalogram underwent a further change with a steady 1 cps slow wave occurring from the left amygdala and hypothalamus and right amygdala (Figure 2h). Here, the animal avoided noxious olfactory stimuli such as liquid dental acrylic and ether, by backing away from them, yet it did follow a visual stimulus, but only to the left. As spikes began to precede the slow waves in the left amygdala, they were propagated to the left hypothalamus and right amygdala when almost simultaneously, bilateral 3 cps spike and slow wave activity began coming from the amygdalas bilaterally (Figure 2i). Concurrently, the cat leaped into the air and began tearing wildly about the cage, growling and hissing, and savagely attacking the walls of the apparatus as if attacking an "imaginary" enemy. Upon provocation with a gloved hand or stick, the growling and hissing became more intense, and the attack was well-directed toward the extraneous stimuli. Associated with this ferocious attack behavior was bilateral facial twitching which eventually developed into a full-blown grand mal convulsion.

Immediately after the convulsion, the cat lay exhausted on its side on the bottom of the cage. The cerebral electrical activity once again assumed a steady 1 cps slow wave from all leads (Figure 2j). As it gradually became attenuated, both the cerebral electrical activity and the heart rate slowly disappeared. This cat died 2 hours after its first bilateral administration of crystalline carbachol.

6. Focal Discharge Without Behavior or Generalized Seizures. To illustrate this specific response to the intracerebral implantation of carbachol crystals as shown in 3 cats (BM-2683, BM-2736, BM-2850), the records of cat BM-2850 will be utilized. The entire pattern of electrical and clinical changes described here took place over a period of 45 minutes. Indeed, it was commonly found with these animals that the electrical and clinical effect lasted for only a short period of time. A characteristic feature of the cats who showed local electrical responses accompanied by the usual focal seizures or automatisms, was that they did not demonstrate an obvious abnormal affective reaction to the chemical stimulation of the amygdala.

Figure 8a shows the precarbachol control recording of bilateral basomedial amygdala and ventromedial hypothalamus placements consisting of the usual low voltage fast activity in the hypothalami and 50 v fast activity mixed with slower frequencies in the amygdalas. Two applications of carbachol 30 minutes apart had no apparent effect. However, 2 minutes after the third bilateral application of 15 μg of crystalline carbachol at the cannula tip, the animal began to sniff at a previously ignored catnip mouse lying in the bottom of the cage. This activity was accompanied by some irregular 50-80 μv theta potentials from the left amygdala (Figure 8b).

A few minutes later, the left amygdala began to show some high amplitude 100-150 μv irregular sharp 6-7 cps complexes, associated with an intent sniffing of the floor of the recording box. At this time, the other placements also began to show brief signs of this unusual activity (Figure 8c),

which was soon followed by a further alteration in which the left amygdalar activity became regular with 80 μv, 6-7 cps theta waves appearing in bursts with each inhalatory sniff at the air (Figure 8d). Traces of the regular theta activity also began to appear in the contralateral hypothalamus.

Gradually, some 50-80 μ v spikes followed by regular 25 cps sharp waves developed in the left amygdala (Figure 8e). Thus, unusual activity was accompanied by rhythmic blinking of the left eye. As both eyes began blinking in unison, a distinct pattern of activity evolved in the left amygdala consisting of 4-second bursts of regular low voltage 30 cps sharp waves followed by 10 cps regular 50 μv activity and ending in 30-100 μv, 20 cps polyspikes (Figure 8f). These bursts of complex activity were followed by 2-3-second interictal periods consisting of low voltage fast activity from all monitored positions. Longer 5-6-second bursts of the previous activity with traces in the ipsilateral hypothalamus and contralateral amygdala and hypothalamus were associated with twitching of the left side of the face.

Rhythmic clonic movements of the entire left side of the body began as these complex bursts became slightly modified to include some regular low voltage 30 cps activity which followed the rhythmic 10 cps sharp potentials. At this time, the ipsilateral hypothalamus demonstrated a much lower voltage variant of this specific pattern of activity.

A few minutes later, the cat suddenly gagged in the middle of a burst of this complex activity. This was immediately followed by an alteration in the pattern of the complicated discharge from the amygdala and hypothalamus with the introduction of some biphasic high voltage 100-120 μv activity

separated by low voltage sharp waves (Figure 8g). This particular change was associated with left-sided body twitching and the animal demonstrating some searching behavior. Related to this behavioral response was the appearance, for the first time, of 150-180 μv spikes from the right amygdala (Figure 8h). Gradually, the left amygdala bursts began to lengthen in duration until they were always associated with high amplitude 200 μv spikes from the right amygdala. Eventually, these long discharges ran together into typical 5-6 cps bilateral spike and slow wave complexes associated with a similar behavioral response to that which accompanied the elicited electrical after discharge (Figure 8i). This consisted of bilateral clonus of 30-seconds duration involving the head and limbs with rhythmic facial twitching, salivation, chewing movements, swallowing movements, and rhythmic clonic movements of all 4 extremities associated with generalized spiking in all subcortical areas.

A few hours following the motor seizure, the electroencephalographic spikes had disappeared and all the monitored subcortical leads demonstrated irregular theta background activity (Figure 8j). At this time, a strong input stimulus such as milk, photic stimulation, petting, or ether could still elicit an abnormal electrical response consisting of bursts of high voltage spikes lasting several seconds from the bilateral amygdalas (Figures 8k-n) without a detectable change in the behavioral response. Twenty-four hours later the record returned to its precarbachol stimulation state and even strong noxious stimuli failed to elicit an abnormal electroencephalographic response or unusual behavior (Figure 8o).

7. Electroencephalographic Response Without Behavioral Change. Three cats (BM-3014, BM-4014, BM-4052) showed an ictal electroencephalographic reaction to the chemical stimulation session with no detectable clinical or affective behavioral manifestations apparent. Characteristically, such animals received four and five bilateral injections of crystalline carbachol before an electrical response was elicited. For example, an alteration in the electroencephalographic record of cat BM-4014 was evident 20 minutes after the fourth bilateral implantation of carbachol crystals. Figure 9a shows a normal control recording taken a few minutes before the first implantation, while Figure 9b demonstrates an amygdalar electrical response to the carbachol drug consisting of moderate amplitude 100 μv irregular theta activity, mixed with the occasional spike and slow wave complex, but were associated with no alteration in the animal's behavioral or electroencephalographic response to affective stimuli.

Other common electroencephalographic responses included irregular sharp activity accompanied by an occasional spike macropotential (BM-3014), or rhythmic bursts of regular low amplitude 30 μv, 7-8 cps sharp potentials (BM-4052). Electrical response signs were extremely short lived, and usually disappeared within 30 minutes of their onset. At this time, the usual sensory and behavioral examination failed to demonstrate any alteration in cerebral electrical activity or the cat's personality.

8. No Electrical, Clinical or Behavioral Change. Three cats (BL-2716, BL-2725, BM-4015) showed no electroencephalographic, clinical or affective reactions to the bilateral implantation of crystalline carbachol

into the basal amygdaloid complex. During the recordings of the chemical implantation sessions, the animals did not seem disturbed in that none of the usual automatisms were present, and the animal behaved as usual.

Administration of the sensory and social behavioral test batteries failed to demonstrate an alteration in the animals' reactions to these affective stimuli. Accordingly, such cats were returned to their home cages in the animal care quarters where they were closely watched during the 24-hour period following the chemical procedure for signs of central nervous system involvement.

A consistent finding with these cats was that they also failed to demonstrate any evidence of a chronic behavioral alteration, although cat BL-2716 was found 6 weeks after the carbachol instillation in rigor mortis. Postmortem examination revealed a massive hemorrhage in the lung with no brain pathology other than that usually associated with the electrode and cannula tracts.

B. Chronic Postictal Behavior

Following intense focal motor or generalized seizures which sometimes ensued for 12-48 hours, a few cats (BM-2778, BM-2902, BM-4016) who survived this severe status epilepticus condition showed ataxia, tremors of the head and/or body, and oriented only to one side. Within the next week, however, they gradually regained most of their previous normal functioning although continuing to show peculiar responses to affective stimuli.

Although the cats appeared fairly normal as they ate and drank voluntarily and superficially responded appropriately to external stimuli,

closer examination revealed that such an animal was in poor contact with its environment in that an underreactivity to all forms of noxious stimuli of moderate intensity was detected. Although the pupils reacted to light, the cat did not avoid the light, nor did it cringe when the observer pretended to strike at it. If smoke was forcefully blown at him, he withdrew somewhat but did not avoid the smoke. A burning match could be brought up to his nose with either no response elicited or actual sniffing and approach that led to burning his whiskers.

With the possible exception of the "no affective reaction" animals, no distinct prediction of chronic behavior was available from the behavioral state associated with the ictal period. Five "no affective reaction" cats (BL-2725, BM-2850, BM-3014, BM-4015, BM-4052) did not show a detectable alteration in their affective behavior during the chemical stimulation session nor were there any apparent chronic behavioral consequences in the weeks that followed the carbachol implantations.

1. Overirritability. This permanent postictal reaction to the crystalline carbachol stimulation of the amygdala bilaterally was seen in 3 cats (BM-2736, BM-2902, BM-4014). Typical chronic irritable behavior manifestations were shown by cat BM-2902 who was representative of this final result. During the ictal phase of the experiment, this cat demonstrated an unusual behavioral response consisting of an alteration between an angry-attack state in which it growled and hissed intermittently and a purring state in which it rolled and purred loudly. Figure 5d demonstrates the electroencephalographic activity related to the angry-attack state

associated with pupillary dilatation, laying back of the ears, hissing, growling, and biting at the observer (Figure 6b). Here, the electroencephalographic record consisted of high amplitude 200 μv, 6 cps spike activity with the amygdalas bilaterally showing the greatest amplitude response. Figure 5e shows the electroencephalographic pattern associated with the hypersexual rolling-purring condition, consisting of high amplitude 150-200 μv, 1-2 cps slow waves accompanied by the occasional spike, seen mainly from the surface motor and occipital cortices, and the subcortical structures (amygdala, hypothalamus, and hippocampus) of the left hemisphere.

Several hours after the chemical activation of subcortical structures, the abnormal spike activity was replaced by low voltage sharp complexes from surface and hippocampal and hypothalamic subcortical leads, while moderate amplitude 80 μv, 5-6 cps theta activity was seen in the amygdalas bilaterally. At this time, the abnormal rolling and hissing behavior was still evident, although it spontaneously occurred at long intervals of 30-40 minutes.

During the next 2 days, the cat developed some signs of permanent irritability. It showed a peculiar hyperreactivity during play with other cats and with humans; biting and scratching were stronger than would normally be the case in this cat. Extremely affectionate with people before chemical stimulation, it later reacted at times to even the slightest touch by growling and assuming the crouched threat posture. This irritable response was totally unpredictable in that at times the cat would purr spontaneously and rub against the observer, while at other times it disliked being handled as evidenced by retreat and struggling to free itself. At this time, strong

olfactory stimulants such as ether resulted in 2-3-second bursts of 5 cps high amplitude 150-200 μv spike activity from the left subcortical electrodes (amygdala, hypothalamus, and hippocampus) and the right amygdala (Figure 5c).

Seven days following the carbachol implantation, the chronic irritability of this cat had stabilized. At this time, the abnormal electroencephalographic response to strong noxious stimuli had disappeared along with the peculiar hypersexual-anger behavioral manifestations (Figure 5g). The cat frequently vocalized and showed other signs of irritability such as hissing and clawing at other cats which it had previously interacted with in a friendly, playful manner. When roughly handled, the cat characteristically hissed, growled, and clawed.

2. Overdocility. Permanent overdocility was demonstrated by 3 cats (BM-2690, BM-2729, BM-2778). Cat BM-2778, illustrative of this reaction, had been pre-experimentally classified as a friendly, playful cat. Carbachol stimulation produced focal motor discharges accompanied by a fear-defense behavioral manifestation (Figure 9a). Figure 9b demonstrates an example of a growling defense reaction which occurred when the cat was threatened with a stick during the acute ictal stage. The focal epileptiform activity progressed to generalized grand mal convulsions within 4 hours of the chemical implantations at which time the animal was placed under a strict schedule of anticonvulsant treatment. Within 36 hours of the onset of generalized seizure activity, the cat had recovered to the point where it was voluntarily eating and drinking, although evidence of severe electroencephalographic abnormalities in the form of 150 μv

intermittent spike activity from all subcortical areas still remained (Figures 9c & d). At that time, sniffing tuna fish increased the intermittent spike activity to 4 cps (Figure 9e), while sniffing an alcohol-soaked sponge elicited a 5 cps spike discharge of a longer duration (Figure 9f) associated with the animal rubbing its face on the sponge and pushing it along the floor with intent interest (Figure 4).

During the first week of recovery from the chemical stimulation session, occasional bursts of epileptiform activity occurred against a background of intermittent spike discharges. These seizures were much shorter in duration than those associated with the acute ictal state, and were discrete, involving slight rhythmic blinking, dilatation of pupils, and movements of the nostrils and upper lip with no salivation.

By the end of 10 days following the bilateral amygdalar chemical activation, the cat had obviously developed much gentler chronic behavioral characteristics. At this time, the animal constantly purred, drooled, and kneaded with its paws while in the company of people. It demonstrated abnormal responses to affective stimuli in that it did not avoid strong light or smoke which was forcefully blown in its face; nor did it cringe when threatened with a blow. The cat consistently sniffed at a burning match, often singeing its whiskers. The animal rubbed up against people and furniture, as well as other cats, totally oblivious to the annoyance of these animals as they growled, hissed or struck out at it. Rough handling or the administration of noxious stimuli, such as tail pinching, failed to elicit previously observed anger reactions.

The electroencephalographic record consisted of low voltage fast activity from the bilateral hippocampal area and the left hypothalamus, while the surface leads and left amygdala showed low voltage sharp activity superimposed on some slower 5-6 cps complexes. The right amygdala and hypothalamus both demonstrated unusual rolling 2-3 cps slow wave background activity with low voltage sharp activity superimposed on it (Figure 9g). At this time, sniffing ether replaced the rolling slow wave activity with irregular moderate amplitude (50-60 μv), 6 cps theta waves (Figure 9h).

Behavior characteristic of overdocility persisted for 3 weeks when the animal suddenly developed symptoms of anorexia and vicious behavior. The new alteration in behavior continued for 3 days, at which time an overdose of Nembutal was administered to the cat, since it was not voluntarily eating or drinking, and its extreme viciousness made it impossible to implement force feeding.

3. Overfearfulness. This chronic behavioral alteration occurred in 2 cats (BL-2723, BM-4016). Cat BM-4016 is typical of this reaction, as it consistently gave the impression of being in a state of apprehension or alarm. Similar to the pre-experimental, quiet, withdrawn animal, the overfearful cat sat far back in its cage and, when approached, withdrew a little, looked about as if in search of an escape route, and was obviously frightened. When the door of the cage was opened, this cat never came out voluntarily, and was always forcibly removed. The cat disliked being petted or carried as evidenced by continued struggling and meowing. When allowed unrestricted movement, the animal would characteristically take flight, run away and hide

when approached by people or by other cats. It often chose a particular hiding place and would seek this place out when confronted.

These cats typically withdrew from all varieties of stimuli, whether pleasant like petting or noxious like tail pinching. When cornered, the cat displayed rather quick and anxious glancing movements, and would cringe and withdraw a little as if prepared to escape from an unknown threat.

Chronic overfearful animals also displayed a peculiar electroencephalographic response to affective stimuli. For example, Figure 3a shows the precarbachol response to an alcohol-saturated cotton ball. Sniffing produced high amplitude 200-250 μv slow waves from subcortical structures, particularly from the right hemisphere. During the midst of a carbachol-induced focal motor seizure (Figure 3b), sniffing an ether ball increased the frequency of amygdalar spike discharge from bursts of 4 cps to a steady discharge of 5-6 cps (Figure 3c). Twenty-four hours later, electroencephalographic activity still demonstrated a steady 1-2 cps spike discharge from subcortical structures (Figure 3d), and sniffing ether increased the amplitude and frequency of the spike activity to 3-4 cps (Figure 3e). At this stage of recovery, calling to the cat also produced a short burst of 10 cps spikes (Figure 3f). Although these abnormal electrical responses to affective stimulation disappeared within 48 hours of chemical injection, the overfearful behavioral manifestations were still present 8 weeks later when the animal was dispatched.

4. No Behavioral Change. Seven cats (BL-2683, BL-2725, BL-2728, BM-2850, BL-3014, BM-4015, BM-4052) showed no permanent postictal

electroencephalographic or affective reaction to chemical stimulation with carbachol. As mentioned above, 5 of these animals (BL-2725, BM-2850, BM-3014, BM-4015, BM-4052) failed to demonstrate an acute alteration in affective behavior during the ictal stage. The remaining 2 cats (BL-2683, BL-2728), which received liquid injections of the drug, displayed short-lived provoked anger-attack reactions that disappeared within 10 minutes of onset as the electroencephalographic activity returned to its prechemical stimulation condition. Thus, all of these cats failed to show a permanent change in behavior in the weeks that followed the carbachol implantations.

III. Electrical Stimulation

A. After-Discharge Threshold Level

All animals with after-discharge threshold level determinations (N = 9: 6 basomedials, 1 basolateral, and 2 controls) generally showed similar response characteristics to electrical stimulation during both pre- and postcarbachol sessions.

At lower levels of stimulation, the first behavioral sign to appear was a change in the ongoing behavior of the cat. Most often the cat would raise its head, perk its ears and stop ongoing movement, in an attention reaction. The patterns elicited by low frequency cathodal pulse stimulation were often associated with stimulus-bound phasic muscle activity, i.e., twitching of the ears, twitching of the whiskers, twitching of the eyes (Figure 10a).

At higher stimulus intensities, other facilitated motor effects synchronous with the stimulus bursts became apparent: pupillary dilatation

which was consistently bilateral and equal, closing the eyes, lowering the ears, retraction of the mouth, rhythmic movements of the upper and lower jaw as in chewing, and rhythmic movements of the nostrils as in sniffing (Figure 10b).

As the after discharges developed in the amygdalas and hypothalamus (which always developed simultaneously), bilateral clonus involving primarily the head and forelimbs developed. This consisted of rhythmic facial twitchings, often accompanied by salivation, rhythmic blinking of the eyes, chewing and licking movements, and hitting and biting at the recording cable or air. The above-described behavioral manifestations were accompanied by repetitive high amplitude 150-300 μv, bi- or triphasic spikes appearing with a frequency varying irregularly from 3-10 cps. The duration of an after discharge following electrical stimulation varied from 4.0 seconds to as long as 45.0 seconds. Usually one amygdala showed the highest amplitude activity, while the ipsilateral hypothalamus demonstrated the next greatest amplitude potentials, followed by the contralateral amygdala and hypothalamus (Figure 11k). The after discharge in the hypothalamus always stopped synchronously with the arrest of spikes in the amygdala.

The precarbachol after-discharge threshold levels of the experimental animals which varied from 1.8 mA in the most sensitive cat (BM-4052) to 2.9 mA in the least sensitive animal (BL-3014), were comparable with the first after-discharge determinations of the control cats which were 2.4 and 2.6 mA (Figure 12).

The results of the second, third, and fourth electrical stimulation of the control animals showed a general tendency for the after-discharge

threshold level to remain constant (Figure 13).

The postcarbachol after-discharge threshold levels demonstrated a general tendency to increase. This was clearly related to the severity of the carbachol-induced convulsions (Table 2), and the degree of postictal personality change (Table 3). Moreover, the after-discharge threshold of animals that suffered generalized convulsions and maximum postictal behavioral change continued to significantly rise 7 weeks after the carbachol ictus. For example, the after-discharge threshold level of cat BM-2850, which suffered only focal motor seizures at the time of carbachol injection, rose to 6.0 mA by the seventh week postcarbachol; while that of Cat BM-2902, which developed generalized seizures and subsequently recovered from the same, rose to 15.0 mA 7 weeks following the carbachol inoculation.

Elicitation of the after discharge by electrical stimulation consistently failed to produce detectable alteration in the personality or behavioral characteristics of the animals. That is to say, the battery of sensory and behavioral tests which was administered following each electrical stimulation, did not demonstrate either a change in cerebral electrical activity or behavioral responses when compared to the results of the same tests given prior to the electrical stimulation procedure. Also, following the electrically induced after discharge, the electroencephalographic activity always returned to its base line within a few minutes.

B. Averaged Evoked Potentials

During electrical stimulation, evoked responses were regularly recorded from the ipsilateral ventromedial nucleus of the hypothalamus following 3 cps electrical pulses of increasing intensity applied to the basolateral or basomedial nucleus of the amygdala. Data from 3 of these cats proved to be acceptable for analysis (BM-2850, BL-3044, BM-4014). Figure 14 demonstrates the square wave of the stimulus signal channel which was used to initiate the 250-millisecond sweep of the Enhancetron; while on the lower channel is the artifact caused by the electrical stimulus as recorded in the ventromedial nucleus of the hypothalamus. The electrical artifact varied in duration from 2.2 milliseconds at low stimulus intensities to 5.0 milliseconds at the higher current levels.

At low electrical stimulation intensities, the hypothalamic ventromedial evoked response was characterized by an initial potential which appeared at long latencies of 5.0 to 10.8 milliseconds. A subsequent shallow potential began about 100.0-120.0 milliseconds after the stimulus (Figures 11a-c). The general character of the evoked response did not alter at higher stimulus intensities, but as the intensity of the stimulus increased, the latency of the response decreased to 1.8 milliseconds after the stimulus and reached peak amplitude between 25.0-30.0 milliseconds (Figures 11f-i). At the current intensity which elicited the electrical and clinical after discharge, the character of the averaged evoked response was altered to show the two unidirectional waves, now of increasing amplitude, each peaking at 25.2 and 73.8 milliseconds, respectively. Thus, the second, smaller response

peaking at approximately 100.0 milliseconds was partially enveloped in the larger initial response at lower stimulus levels, but now reached a maximal amplitude.

Three records of this type obtained from the 3 cats demonstrated the consistency of this response in different animals. A single difference between basolateral and basomedial stimulation was that the averaged evoked hypothalamic response was smaller in amplitude when induced by basolateral amygdala pulses.

In chemical stimulation, the hypothalamic averaged evoked response also looked similar regardless whether the amygdala spike originated basolaterally or basomedially (Figures 2a-j and 8a-i). The response of the ventromedial nucleus to the chemically induced amygdala spikes gradually became apparent as the ictus progressed. Initially, the evoked response occurred approximately 20.0 milliseconds after the amygdala spike (Figures 2b-d), but as the response of the hypothalamus on the electroencephalographic record increased in amplitude, it occurred simultaneously with the amygdala spike (Figures 2e-j). As the amplitude of the amygdala spike increased, the character of the averaged evoked response was altered from an initial large amplitude response followed by a shallow potential about 54.0 milliseconds after the amygdala spike, to one maximal response per 250 millisecond epoch.

IV. Histology

Histological study of the brains of 15 experimental and 2 control animals showed that the cannula tips were all located with the confines of the amygdaloid complex, the majority within their appropriate basolateral or basomedial

subdivision. Cats BM-2683 and BL-3044 had cannulas within the anterior area and corticomedial subdivision, respectively. A typical placement in the basomedial amygdaloid complex is shown in Figure 15.

Microscopic examination of the amygdala cannula tracts in 15 experimental and 2 control animals revealed a destruction along the needle tract without neural elements but with polymorphonuclear cells, capillary proliferation and collagen tissue. Around the tract there was a rather delimited capsule with abundant fibroblasts, collagen tissue and some neuroglia and microglia cells. The size of the tract varied, the usual diameter being about 1 millimeter. Beyond the capsule neurons appeared well preserved in general.

Histological study of the points which were repeatedly electrically stimulated in the 2 control cats did not reveal any special characteristics. For example, an amygdalar section from cat BM-4022 demonstrates the region of necrotic tissue within 1.0-1.5 mm of the tip of the cannula (Figure 16). On the other hand, examination of the same subcortical region in animals that received chemical implants, whether or not they demonstrated chronic behavioral alterations, revealed a maximum region of tissue destruction extending 2.8-5.0 mm from the site of chemical deposition at the tip of the basal amygdaloid cannula (Figure 17).

Cellular destruction and fiber tract degeneration in the area of the carbachol deposit apart from the usual 1.0 mm lesion at the tip of the amygdala cannula, can probably be attributed to the direct mechanical and chemical effects of the epileptogenic material.

DISCUSSION

The major goals of the present investigation were to determine: (1) whether the chronic rage, reported by Grossman (1963), following carbachol induced seizures of the basal amygdaloid complex of the cat was due to a chronic discharging epileptogenic (hyperirritable) focus, or to a deficit (hypoirritable) lesion at the initial site of chemical stimulation; and (2) whether cholinergic stimulation so induced caused activation of different regions in the amygdala and its projections than electrical stimulation.

Grossman (1963) reported that a single injection of crystalline carbachol into the basal nuclei of the amygdaloid complex produced generalized epileptiform seizures followed by striking behavioral and electrophysiological changes which persisted indefinitely. Following recovery from severe and prolonged seizures of the immediate postcarbachol period, the cats were so vicious that they could not be handled for further experimental work. At this time, high voltage electrical spike activity from the amygdala and hypothalamus, characteristic of the ictal state, was permanently changed to a low voltage fast pattern with scattered random spike activity. Both electroencephalographic and behavioral changes persisted until the animals were sacrificed 5-6 months following the injection of 5-10μg of carbachol bilaterally.

None of the cats in this study developed the dramatic postictal rage state described by Grossman. However, of the 21 cats injected with carbachol in the basolateral and basomedial amygdala, only 3 animals survived the severe, generalized convulsions that always preceded postictal viciousness in the Grossman cats. Moreover, none of the animals reported here survived generalized

convulsions produced by basolateral injections of carbachol (the site implicated in the original report by Grossman [1963], although not born out by inspection of an original protocol forwarded from his laboratory). None of the 3 animals surviving generalized status from basomedial injection became savage, although one was moderately hyperirritable, another hyperdocile, and a third hyperfearful. All 3 cats had altered postictal electroencephalographic changes from the amygdala and hypothalamic regions, but in contrast to the Grossman result of low voltage fast activity, slow, low voltage potentials persisted from the amygdala and hypothalamic site, with hypersynchronous spikes appearing only upon provocation. These animals all showed striking after-discharge threshold elevations. Thus, the findings from this study indicate that a depression rather than a facilitation of the injected area ensued following recovery from the generalized seizures.

Three cats developed generalized seizures within 2-12 hours following carbachol deposition and died within the subsequent 36 hours, evidently from status epilepticus or treatment of same. At least one of these animals was shown to be in status epilepticus from subcortical regions only, even though behavior was that of rigid catatonia. Three others were found dead in their cages 12-14 hours after apparent recovery from immediate postcarbachol generalized seizures. It is assumed that they too succumbed to recurrence of status epilepticus or its lethal aftereffects.

In contrast to Grossman, who reported generalized seizures in all animals and postictal rage in every cat that survived the seizures, 3 cats in this study showed no electrical or behavioral response to implantation

of carbachol in amygdala; 6 others demonstrated only autonomic or focal motor seizures; and 9 animals manifested short-lived behavioral patterns of defense, attack, and escape during the carbachol induced ictus. In agreement with Grossman, all 3 survivors of generalized seizures had permanent behavioral change, while only one of the other animals (with carbachol-induced focal seizures) did so. As noted above, none of these 4 animals were in any sense savage or hard to handle.

A number of possibilities exist which may explain the discrepancies between the present study and that reported by Grossman. It may be that the structures chemically stimulated in both studies were not strictly identical, since Grossman speaks of a basolateral amygdala inoculation of carbachol in his original communication (Grossman, 1963), but his protocols indicate a more basomedial stimulation site corresponding to A- +12.5, L- $\pm$9.0, and V- -6.0.[1] Both basolateral and basomedial carbachol injections were used in this series of experiments, but no cat survived generalized seizures induced by the basolateral injection.

A total of 9 cats were implanted with basolateral amygdala cannulas. One displayed violent rage and a single generalized seizure within 2 hours of chemical injection followed by exhuastion, cardiac irregularity, and death preceded by a flat electroencephalographic record. This animal received, as nearly as was possible to duplicate, the same amount of carbachol used by Grossman. Three other animals expired within 48-72 hours of the onset of status epilepticus which in two instances succeeded ictal anger and fear-defense responses. Thus, no basolateral animal survived status epilepticus.

[1]Personal Communication, 1970.

Of the remaining 5 basolateral animals, 2 showed spontaneous ictal anger-attack behavior followed by return to normal behavior within 24-48 hours. One cat displayed neither ictal nor postictal behavioral alterations; another demonstrated only an alteration in electrical activity following carbachol, and no immediate or long-term behavior change. A final cat demonstrated ictal fear-escape responses followed by moderate postictal docility. Thus, although one basolaterally injected cat displayed a permanently altered personality, none of these cats showed any signs of chronically enraged behavior.

Pathological examination of the basolateral animals revealed similar 3.0-5.0 mm^2 areas of necrosis at the amygdala cannula tip in all injected cats, but no evidence of degeneration in hippocampus, hypothalamus, or other subcortical structures (beyond that associated with the recording electrode tracts).

Twelve cats received carbachol through basomedial amygdala cannulas placed according to the coordinates listed on Grossman's original protocols. Two of these animals died in general seizures within 2 hours of the carbachol deposition with one cat displaying several hours of savage, violent, attack behavior accompanied by piercing caterwauling. Another 3 animals, one of which showed no behavioral alteration associated with the acute ictal state, and 2 of which displayed either provoked fear-defense or anger-attack reactions, developed generalized seizures within 12 hours of chemical injection. All 3 of these animals were treated with luminal during ictus, and survived with chronic personality alterations of hyperfearfulness, hyperdocility,

and hyperirritability, respectively, accompanied by local slowing or spike activity in the electroencephalogram, and increased after-discharge threshold levels at the site of chemical injection.

Two cats displaying either ictal fear-escape or anger-attack reactions associated with focal evidence of electrical ictus in amygdala, hypothalamic and hippocampal structures, lateral developed chronic behavioral characteristics of moderate docility; while 2 other animals displaying either focal motor seizures or focal electrical spike activity in the amygdala, hypothalamus and hippocampus immediately following carbachol instillation developed chronic hyperirritability in the postcarbachol period associated with a moderate increase in after-discharge threshold level. The remaining 3 animals were unchanged during the immediate or chronic postcarbachol injection period, and showed the least elevation of the after-discharge threshold level (except for the control animals). Histological examination revealed similar localized necrosis at the cannula tips in all animals and did not explain the different postcarbachol outcomes.

In summary, 3 animals of the basomedial group survived generalized status epilepticus, and all of these cats demonstrated chronic behavioral alterations. One of the 6 animals with focal seizures also developed a chronic behavioral change. Postictal rise in the after-discharge threshold was greatest in animals suffering status epilepticus, and continued to rise 7 weeks following carbachol implantation; was least in the animals which developed neither behavioral nor electrical changes after chemical deposition; while cats suffering focal seizures occupied on intermediate position.

Control cats demonstrated no significant change in after-discharge threshold level in the 4-week postoperative period.

Thus, although status epilepticus was associated with a permanent alteration in behavior, it did not lead to chronic rage in the basomedial cats. It does appear, however, that the chronic behavioral alteration is related to the severity of the initial convulsions, and in a subsequent change in after-discharge threshold of the amygdaloid and hypothalamus. The progressive rise in the after-discharge threshold probably reflects cellular degeneration or other alterations in structures at the site of the injection and possibly in projection areas as well.

A second difference may be in the amount of carbachol injected into basal amygdaloid region. Grossman (1963) reported delivering minute quantities of 2.0-5.0 µg of crystalline carbachol, an amount which in this study was found to be impossible to measure, or to produce observable results. Accordingly, following the procedure of Baxter (1967, 1968), individual doses estimated to be 15.0-25.0 µg were utilized. As previously mentioned, when these amounts failed to evoke a reaction, whether electroencephalographic or behavioral, additional carbachol was administered bilaterally every 30-60 minutes until an electrical seizure was induced or the total amount of crystalline carbachol injected reached 65.0-70.0 µg without inducing any result. In this study, 4 of the 8 animals that demonstrated a mild to moderate permanent alteration in their behavioral state received bilateral injections of 50.0-65.0 µg of crystalline carbachol; while 5 of the 10 cats which showed no behavioral or electroencephalographic change received

identical amounts of carbachol. Thus, dosage of carbachol does not relate to the final behavioral outcome.

Although MacLean (1957a) and Grossman and Stumpf (1969) indicate little diffusion of the crystalline drug within an hour of inoculation, it is possible that the large dose used here to obtain observable results may have undergone widespread diffusion from the site of deposition. In fact, a number of investigators have criticized the technique of stimulating the brain with crystalline chemicals on the basis of a progressive slow diffusion to other subcortical structures, including the ventricles (Baxter, 1968; Routtenberg, 1967).

Grossman reported that microscopic examination of the histological material from his experiment did not reveal evidence of tissue damage greater than that produced by mechanical damage due to the cannula itself. However, although pathological examination was generally limited to the identification of cannula sites and an evaluation of major histological changes, it was regularly found in the present study that local tissue destruction was more extensive in cats that received bilateral implantation of carbachol than in control animals with cannulas alone. This finding suggests that the drug implant damaged tissue not only at the cannula tip but up to 5.0 mm of the surrounding region as well by diffusion from the site of deposition. It is also possible that the larger dose used in this study in order to obtain observable results may have permanently depolarized or damaged the brain tissue surrounding the site of the chemical implant. Miller, Grottesman, and Emery (1964), in a study of drinking

behavior in rats using liquid carbachol and norepinephrine as chemical stimulants, found inverted U shapes of the dose-response curves in that they failed to obtain positive results either by using too high or too low a dose. This latter finding may also explain why no electroencephalographic or behavioral changes were observed in 3 animals, all of which ultimately received rather large doses of intracerebral crystalline carbachol. However, when special care was taken with 2 final animals to deliver exactly the same dosage as Grossman (3 taps of a 26-gauge needle on a watchglass containing carbachol), one cat developed fatal status, while the other demonstrated neither an electrical or behavioral change. Pathological examination demonstrated similar cannula placements in the basolateral nucleus of the amygdala in both animals with similar histological changes.

In the series of animals presented here an attempt was made to duplicate Grossman's procedure in every way except one, i.e., it was not possible to use free-born, outdoor alley cats as Grossman did. All the animals used in this study were born in a special animal breeding farm and spent their entire lives in cages. The importance of this factor is not known and cannot be assayed by the animals utilized here. It is of interest to speculate that the strain or past history may have influenced the ability of the animal to survive the severe stress of generalized seizures or the infrequent consequent behavior.

Ursin and his associates (1960, 1965a & b) have distinguished three patterns of emotional behavior in the cat following electrical stimulation

of the amygdala: (1) defense, (2) attack, and (3) flight. Electrical and chemical stimulation, as well as ablation studies, have shown that all three behavior patterns appear to have separate, although somewhat overlapping, representations in the amygdaloid complex (Goddard, 1964; Ursin & Kaada, 1960; Wood, 1958).

Goddard (1964) has contended that within the amygdala there appears to be considerable intermingling of the anatomical substrates for flight and defense mechanisms. Ursin (1965a) attempted to show that two analogous response elements, flight and defense, have separate control regions within the amygdala. However, he too found that within the amygdala there appears to be an extensive intermingling of the anatomical substrates for both types of behavior.

Previous studies (Gloor, 1955a, 1955b) have shown that the subcortical amygdala projection field is a complexly organized network of neurons with a variety of functional properties that are apt to show a prominent increase of their excitatory state under the influence of repetitive amygdaloid bombardment. Moreover, potential mechanisms of enforcement of excitation are given by the confluence of alternative pathways of the direct and indirect amygdalohypothalamic fiber tracts upon common neuronal pools in the septum and hypothalamus, some of which are relayed through multiple synapses in the amygdala, suggesting an intra-amygdala association system, and others through the hippocampus which discharges into the hypothalamus via the fornix.

Extensive functional overlap of opposing affective systems in the amygdala could explain the considerable variability in the responses elicited

from the animals in this study. The reactions evoked from these animals support Goddard's contention of a lack of geographic functional differentiation of emotional behavior in the amygdala, and indicates that the chemical utilized here probably influenced more than one functional substrate.

The dissociation between the ictal and postictal state, as demonstrated in 8 cats with postinjection chronic behavioral change suggests that different neuronal mechanisms were responsible for behavior associated with the postictal period.

The ictal behavior of the 8 cats included components of fear behavior (moving about wildly in all direction, escape behavior), defense reactions (biting of recording cable, striking out with paws when directly confronted), and attack reactions (baring of the teeth, growling, snarling, biting and grasping the experimenter's gloved hand if threatened).

Several features of chemically induced after discharge may singly or together explain the change in affective response during the ictal stage. First, the propagation of ictal discharge to other limbic structures was directly related to the length of the chemically induced spike discharge. Second, if in any way the affective response depended on the number of amygdaloid neurons discharged, then this number might be increased by the local hypersynchrony of the sustained discharge. Thus, the ability of an amygdaloid discharge to produce an affective response may depend finally on the recruitment of neurons in hypothalamic and other limbic structures. The repetitive and hypersynchronous neural activity of the after discharge

may facilitate such recruitment. It is well known that chemical stimulation of the ventromedial hypothalamus alone can produce affective responses (for example, fear, defense, attack) similar to those accompanying an after discharge in the hypothalamus elicited from amygdala stimulation. However, direct hypothalamic stimulation need not elicit an after discharge to produce such behavior. It is likely that hypothalamic stimulation directly exictes the "final common path" that elaborates that response.

There is evidence that axons of basal amygdaloid neurons reach the ventromedial nucleus of the hypothalamus only after several synapses (Hall, 1965; Nauta, 1961; Raisman, 1970). Progressive increase in stimulus intensity may excite these final neurons through synaptic resistance. The sustained excitation provided by the potent cholinergic agent carbachol effectively provides this increased stimulus intensity.

In all cases, ictal alteration in behavior produced by chemical stimulation was shown to depend on activation of sustained hypothalamic spike activity. The postictal changes in affective behavior occurred only in animals that developed spike activity in the hypothalamus, as well as the amygdala. The specific characteristic behavioral alterations of the postictal period were not regularly predictable from the ictal behavioral manifestations or the preictal personality (Table 1).

The importance of the hypothalamus and amygdala in emotion has been shown by numerous investigators. Many studies have implicated both the hypothalamus and amygdala in the integration of aggressive behavior. With

respect to the hypothalamus, the evidence indicates that the ventromedial hypothalamus contains an important integrating center for the expression of emotional behavior, particularly for anger and aggression (Bard, 1928; Nakao, 1958; Wasman & Flynn, 1962).

Because of its rich connections with many subcortical structures, including the hypothalamus, several investigators have suggested that the chief function of the amygdala is a modulatory one, modulating in particular the activity of the hypothalamus. For example, Bard and Mountcastle (1948) suggested on the basis of physiologic evidence that "the area of the amygdala acts as a funnel through which inhibitory influences originating in the transitional cortex of the midline, in the neocortex, and in the amygdala itself exert a suppressing action on brainstem mechanisms," and Gloor (1955b) concluded the report of his extensive electrophysiological studies with the statement that "the amygdala is thought to act in a modulatory way in complex somatic, autonomic and behavioral mechanisms integrated in subcortical structures".

Direct and most important evidence that amygdaloid activity can influence the electrical activity of the hypothalamus has been presented by Eleftheriou, Church, Zolovick, Norman, & Pattison (1969). They demonstrated that electrical stimulation in the amygdala can influence the rates of firing of single units in the hypothalamus. Another important line of evidence that amygdaloid activity can influence behavior elicited by the hypothalamus stems from the experiments of Egger and Flynn (1962, 1963). These investigators showed that discrete areas of the amygdala inhibit while others facilitate rage-like attack behavior induced by direct stimulation of the hypothalamus.

Kaada (1951) was the first to propose that the amygdala contained inhibitory neurons that hold the ventromedial hypothalamic aggression-defense center in check. The evidence presented by Kaada and by others (Wood, 1958) indicates that very discrete lesions in specific amygdalar regions elicit rage by avoiding simultaneous or prepotent stimulation of an inhibitory mechanism. Similarly, unless lesions are very discrete or specific, simultaneous destruction of defense and flight areas gives unpredictable results.

It appears, then, that there exists a small area within the amygdala exerting an inhibitory influence on aggressive behavior. Since the result of total amygdalectomy is increased tameness, the influence from such an inhibitory zone must be postulated to be weaker than that of the excitatory zone. A lowered threshold to rage-provoking stimuli might, therefore, be unveiled only by bilateral lesions confined to this restricted subdivision of the amygdala.

In the cat the amygdaloid complex is a very compact area with maximum dimensions of less than 1.0 cm. From stimulation experiments, a rage locus might preferably by expected in the medial group of nuclei. This appears to be the optimal region for the inhibitory effects on reflex and cortically induced movements (Kaada, 1941), as well as on blood pressure and respiration (Kaada, 1951; Wood, Schottelius, Frost, & Maitland, 1958). The latter effects have been shown to be funneled through the ventromedial hypothalamic area, since they were abolished after bilateral destruction of this region (Gloor, 1960).

Grossman's experiments (1963, 1964, 1970) suggest that his carbachol lesion either permanently and selectively depressed a rage inhibitory network projecting to the ventromedial nucleus of the hypothalamus or rendered a facilitatory component from the amygdala to the amygdala to the hypothalamic region hypersensitive. Since the chronic rage always followed generalized seizures, it is possible that the selective destruction of inhibitory terminals or hypersensitivity of excitatory neurons may have been conferred "down stream", i.e., on the hypothalamus or hippocampus following prolonged stress and local tissue anoxia or other metabolic derangements.

MacLean (1955) postulated that the intense seizure activity produced by the local injection of carbachol selectively abolishes inhibition. In addition to inhibition of hypothalamic neurons subserving rage, this type of inhibitory suppressor mechanism has been proposed by other investigators to explain amygdala control of food intake. Lewinska (1967) concluded from his study on the functional relationship between the ventromedial nucleus and the amygdala that the ventral portion of the ventromedial nucleus is inhibited by the amygdala with respect to food intake.

The fact that either hyperdocility, hyperirritability or hyperfearfulness followed prolonged ictus in this study, indicates a relatively selective ablation or depression of either facilitatory or inhibitory systems causing the animal to handle incoming stimuli in a new manner. This interpretation is supported by the rise in the after-discharge threshold level following ictus, indicating decreased local excitability at the cannula tip and possibly in the hypothalamus as well and with the altered electrical response

of the hypothalamus to sensory affective stimuli, suggesting loss of modulation.

The after-discharge threshold level determined in 2 control animals failed to demonstrate a difference between the first ($\overline{X}$ = 2.5 mA) and fourth week ($\overline{X}$ = 2.55 mA) of electrical stimulation. Cats which suffered generalized convulsions and status epilepticus showed a definite increase in threshold level between the first week prechemical determination level ($\overline{X}$ = 2.05 mA), and the postchemical electrical stimulation carried out at 3 weeks ($\overline{X}$ = 4.1 mA) and 7 weeks postcarbachol ($\overline{X}$ = 15.0 mA). After-discharge threshold levels of cats that displayed an enduring change in their personality states showed a progressive increase in after-discharge threshold during the weeks following the central chemical stimulation. In fact, the after-discharge threshold of one such animal 7 weeks following the carbachol implant demonstrated a 7-fold increase from the prechemical stimulation state. After-discharge thresholds from animals which displayed focal motor seizures rose from a prechemical stimulation mean of 2.8 mA to a postcarbachol level of 3.4 mA at 3 weeks and 6.1 mA at 7 weeks; while other animals which received carbachol implants, but demonstrated no consequent ictus or behavioral alteration also tended to increase but at a relatively slower rate and to a lesser degree. For example, the mean precarbachol after-discharge threshold level of these animals was 2.5 mA, while the same determination made at 3 and 7 weeks was 4.7 and 5.5 mA, respectively.

Thus, it appears that in all animals in which the basal amygdaloid region was inoculated with crystalline carbachol, there is evidence of

depression or destruction of excitable elements but that the degree to which excitation is decreased is related to the occurrence of focal and, in particular, of generalized seizures, not to the dose of carbachol delivered. If the region of the amygdala chemically stimulated in this study normally subserves both inhibitory and excitatory functions over the aggressive-defensive functions of the ventromedial nucleus of the hypothalamus under standard circumstances, then depending on the relative destruction of excitatory and inhibitory systems induced by the local chemical injection, different degrees and qualities of chronic behavioral alteration would be anticipated.

Concerning the chronic postictal behavior of the cats in this study, it is possible that the intense epileptic seizure activity which followed the intracerebral injections of carbachol, induced lethal anoxic effects both at the site of the epileptogenic focus and the hypothalamic and hippocampal neurons so affected (Crowell, et al., 1968). In contrast to Crowell and his associates, pathological evidence for gross histological change in the animals here was not forthcoming. However, death in, or immediately after, status epilepticus in 6 cats supports such a hypothesis. Wood (1958), on the other hand, demonstrated degeneration in the ventromedial nucleus of the hypothalamus after lesions in the central amygdala nucleus and, as is well known, discrete lesions of the ventromedial nucleus can also induce chronic rage (Schreiner & Kling, 1953).

Whatever the pathophysiologic mechanism of excitants such as carbachol, it appears likely from this study that the chronic behavioral abberations

seen here are directly related to the involvement of subcortical nuclei in the epileptic discharge, the change subsequently shown in the after-discharge threshold, and the altered electroencephalographic activity from these regions.

As is well known, behavioral alterations can be produced by both small and large lesions limited to the medial aspects of the temporal lobe. If the lesion involves an extensive area of the amygdaloid complex, as it did in the present study, and thus both excitatory and inhibitory structures are influenced to an unequal degree, then the quality and severity of the final behavior change reflects both the region involved and the extent of the destruction which occurred. However, despite the existing damage to the basal amygdaloid, the present animals began to react more normally, as epileptiform electroencephalographic activity subsided. The problem then becomes one of homeostasis, the balance of the remaining inhibitory and excitatory influences being most important. It is during and immediately following the ictal stage of the carbachol action that this new dynamic homeostatic relationship is evolved.

It is, of course, impossible to link with certainty the changes in electrical threshold with specific mechanisms in the neurochemistry of affective behavior; however, it is interesting to speculate that perhaps the unbalancing of the neurophysiologic mechanisms of excitation and inhibition as suggested by the increased after-discharge threshold level, is also reflected neurochemically in terms of either a depression or elevation of the catecholamine or cholinergic system (Ervin, Mark, &

Stevens, 1969). In the total functioning organism, then, these neurophysiological and neurochemical systems would probably be in some kind of dynamic balance.

One of the aims of the present study was to compare the behavioral, electroencephalographic, and evoked potential responses elicited by both electrical and chemical stimulation of the basal amygdala in order to establish whether these two methods of brain investigation affect similar or different physiologic substrates.

The behavioral, autonomic and skeletal responses to both electrical and chemical stimulation of the basal amygdaloid complex revealed some similarities, in that salivation, pupillary dilatation, piloerection, licking, chewing and swallowing movements, rhythmic twitching of the ears and blinking of the eyes, and focal facial movements were elicited by both techniques. MacLean (1954), in an earlier study, called attention to the fact that the seizure activity induced by carbachol bears many similarities to that of after discharges elicited by electrical stimulation.

In the present study, a common characteristic of such responses was that they endured throughout the duration of the after-discharge seizure whether chemically or electrically induced. Also related to this last point, is that during the chemical activation of abnormal electroencephalographic activity, it was possible to elicit both pathological electrical and/or behavioral responses to affective sensory stimuli. However, once the cerebral electrical activity had returned to its prechemical or pre-electrical stimulation state, no abnormal electroencephalographic or

behavioral reaction could be elicited. Thus, it appears that the characteristic acute reactions to both stimulating procedures occur only during the period of altered brain activity.

Although both the initial reaction which consisted of an immediate cessation of ongoing activity, and the appearance of an alerting response which included perking of the ears and looking around as though puzzled, and the above-described autonomic and skeletal reactions which occurred as the cerebral electrical activity progressed towards an after-discharge level, were similarly induced by both techniques; the total behavioral pattern produced by each procedure showed some basic differences. In general, electrical stimulation elicited a number of simple response elements which appeared to be components of more complex behavioral patterns, while the reactions evoked by chemical stimulation greatly resembled the purposive responses of a cat. After the initial alerting reaction, the chemical stimulation procedure usually produced two specific responses peculiar to that technique, whether the animal was injected basomedially or basolaterally. The first response was a sniffing reaction which may suggest activation of the corticomedial olfactory region of the amygdala; while the second response, which occurred within a few minutes of the first reaction, consisted of either visual searching movements or fixed staring which may suggest involvement of fibers from the optic tract (Gergen & MacLean, 1964).

These two reactions provide a clear distinction between the development of chemically induced and electrically induced behavioral alterations. That is to say, the occurrence of the specific chemically evoked reactions initiates

the onset of more complex behavioral patterns. As the chemical discharge persisted and spread to hypothalamic, septal and hippocampal regions, specific affective behavioral responses developed. These included growling, hissing, fearful withdrawal, wildly attacking of the sides of the observation box, catatonia, etc.

Thus, a comparison of the electrically and chemically induced behavioral correlates suggests that initially, both types of stimuli were probably affecting similar specific physiologic neural response circuits in the amygdalar region. However, as the intensity, duration, and distribution of the chemically induced discharge increased, complex dynamics, purposive behavioral patterns were evoked, which were in marked contrast to the fragmentary generally nonaffective responses associated with electrical stimulation and the consequent after discharge.

To further examine differences between the results of chemical and electrical stimulation of an identical amygdala site, the averaged evoked response from ventromedial nucleus of the hypothalamus to variable, brief subthreshold electrical pulses was compared with that from repetitive single spikes which followed carbachol.

When electrical pulses were of low intensity and carbachol spikes were small in amplitude, the hypothalamic ventromedial evoked response appeared at latencies of approximately 10.8 milliseconds to electrical stimuli and 25.2 milliseconds to the carbachol induced spikes. The substantial length in the latencies of these evoked potentials in the hypothalamus suggests that more than one synapse was involved. With higher electrical stimulus

intensities and larger amplitude carbachol spikes, the latency shortened to approximately 1.8 milliseconds for both procedures. These results suggest that initially, electrical and chemical stimuli stimulated different elements in that the low stimulus strengths excited the ventromedial neurons synaptically, whereas at higher intensities, neurons of the hypothalamus were also excited antidromically via the stria terminalis and other tracts coursing from the ventromedial hypothalamic region to the amygdala. The delayed synaptic activation presumably does not appear with the stronger stimuli because of the powerful postsynaptic inhibition following antidromic invasion (Ball & Shepherd, 1968; Nicoll, 1969).

The gross evoked response underwent obvious alteration in its characteristics as the stimulus intensity increased or the carbachol spikes increased in amplitude (Figures 2a-j and 11a-j). The morphological differences between the electrically and chemically evoked hypothalamic responses also indicate that chemical and electrical stimulation procedures affect different physiologic substrates.

SUMMARY AND CONCLUSIONS

According to Grossman (1963), the injection of minute quantities of the long-acting cholinergic stimulant, carbachol, into the basal amygdaloid region of cats' brain produced long-lasting functional changes which were reflected in chronic aggressiveness and which he attributed to permanent facilitation of the amygdala rage center.

In the present study, an attempt was made to learn whether or not these lasting behavioral and electroencephalographic changes obtained by chemical stimulation of the basal amygdaloid complex were due to an irritative epileptogenic focus or to the destruction of the nerve tissue at the site of chemical stimulation; and whether chemical stimulation so induced, caused activation of different regions in the amygdala and its projections than electrical stimulation.

Careful behavioral and electroencephalographic observations were made during and following electrical and carbachol stimulation of the amygdala in waking cats with chronic implantation of insulated cannulas and recording electrodes. Affective reactions to a variety of peripheral sensory stimuli were assessed by quantified behavioral tests prior to and following electrical and chemical stimulation procedures.

Six animals died during or following severe seizures which followed the implantation of carbachol bilaterally into the basolateral or basomedial amygdaloid complex. In animals which survived seizures, no state of intractable viciousness was produced. However, all cats which survived generalized

seizures demonstrated some chronic behavioral change. Chronic behavioral alteration was seen in 4 cats and was evidently related to the severity of the initial convulsions and a substantial increase in the after-discharge threshold level of the basal nuclei of the amygdala and/or hypothalamus. Although all carbachol implanted cats demonstrated large lesions at the site of carbachol instillation, the histological data failed to demonstrate evidence for pathological changes in the amygdala related specifically to the chronic postchemical personality alterations.

In summary, from the after discharge and histological data of the present experiments, it seems reasonable to conclude that the chronic affective behavioral manifestations demonstrated here were primarily due to the effects of the initial convulsions produced by the carbachol implantation.

Although electrical stimulation produced a more reliable behavioral and electroencephalographic activation pattern, chemical stimulation with carbachol elicited full-blown, sustained, autonomic and behavioral sequences of dramatic intensity and ferocity which were succeeded by lethal status epilepticus or death, even in the absence of generalized or intense focal seizures. It appears then from the behavioral and electroencephalographic evidence presented that neither electrical nor chemical stimulation procedures permit selective activation of specific functionally defined neural substrates, since both techniques led to the concurrent activation of a number of individual physiologic and behavioral systems.

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APPENDIX 1. Behavioral rating scale used to examine cats' postsurgical, pre-electrical and chemical, and postelectrical and chemical behavior.

Behavior Test

at #: Date: Test #: Time:

Test	Rating 1	2	3	4	5	EEG
ual rception easant oxious						
faction leasant oxious						
tile easant oxious						
dition easant oxious						
ts R to other Cat						
ts R to g held						
uedhand oduced box						
t nip roduced						
dosis R						
	+ Approach	± approach	neutral	± avoid	+ avoid	

APPENDIX 2[1]

Glossary of Electroencephalographic Terminology

Activity: Any sequence of waves.

Attenuation: Decrease in amplitude of activity.

Background Activity: More or less general and continuous activity, in contrast with paroxysmal and focal activities.

Bilateral: Occurring on both sides of the head.

Burst: Group of waves which appears and disappears abruptly and which is clearly distinguished from background activity by different frequency, morphology or amplitude.

Complex: Group of two or more waves, clearly distinguished from background activity and occurring with a well recognized form or recurring with consistent form. Example: "Spike and wave complex".

Cycle: The complete series of potential changes undergone by a wave before the same series is repeated.

Diffuse: Occurring over large areas without constant location. Is used to describe activity occurring more or less simultaneously without necessarily being synchronous in large areas.

Diphasic: Wave deflected first to one side then to the other of the base line.

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[1]Adapted from: Van Leeuwen, W.S., Bickford, R., Brazier, M., Cobb, W.A., Dondey, M., Gastaut, H., Gloor, P., Henry, C.E., Hess, R., Knott, J.R., Kugler, J., Lairy, G.C., Loeb, C., Magnus, O., Daurella, L.O., Petsche, H., Schwab, R., Walter, W.G., & Widen, L. Proposal for an EEG terminology committee of the international federation for electroencephalography and clinical neurophysiology. EEG Clin. Neurophysiol., 20, 1966, 293-320; and Sannit, T., Lilienthal, E., Bevilacqua, J.E., Silverman, D., & Tarlau, M. Glossary of medical terms used in electroencephalography. Amer. J. EEG Technol., 3, 1963, 31-49.

Driving: Occurence of waves phase-locked with rhythmic stimuli.

Electroencephalogram (EEG): Record of electrical activity of the brain.

Electrical Silence: Absence of electrical activity.

Focus: A limited region involved by, or the point of maximum potential of, a specified wave or activity.

Frequency: The number of complete cycles of a rhythm in one second.

Low Voltage EEG: EEG in which no activity larger than 20 V can be recorded between any 2 points on the scalp.

Monophasic: Wave deflected to one side of the base line.

Montage: Combination of a number of pairs of leads or electrode placements.

Morphology: The shape of a wave or activity.

Paroxysm: A group of waves which appears and disappears abruptly and which is clearly distinguished from background activity by different frequency, morphology or amplitude.

Sharp and Slow Wave Complex: Complex of two waves, one having a duration between 1/12 and 1/5 second, the other between 1/2 and 1 second.

Sharp Wave: Distinguished from background activity, with a duration of more than 1/12 and less than 15 second, with rapid rise and fall time.

Slow Wave: Wave with a duration of more than 1/8 second. Slow waves, therefore, include theta and delta waves.

Spike: Wave distinguished from background activity and having a duration of 1/12 second or less, rising and falling more rapidly from base line than a sharp wave or two waves of similar duration.

Spike and Wave Complex: Complex of two waves, one with a duration of 1/12 second or less ("spike") and the other with a duration of 1/5-1/2 second ("wave").

Poly-Spike and Wave Complex: Spike and wave complex with more than one spike.

Theta Activity: Series of regular or irregular waves with a frequency of 4-7 c/second and durations of 1/4 to more than 1/7 second.

Transient: Any single wave or brief complex, notably different from background activity.

Unilateral: Occurring on one side of the head.

Wave: Any transient change of potential difference in the EEG.

APPENDIX 3

Action of Carbachol

Concerning the possible mechanism of action of carbachol, it seems pertinent to review the proposed mechanism of action for acetylcholine (ACH), since it is generally well accepted that carbachol mimics the action of ACH. It has been postulated that ACH may act by increasing the permeability of neurons to sodium, thus being related to the action of the sodium-potassium pump (Fatt & Katz, 1951; Takeuchi & Takeuchi, 1960). Carbachol has been considered to act by release of ACH at vascular smooth muscle neuroeffector junctions and in sympathetic ganglia (Renshaw, Green, & Ziff, 1938; Volle & Koelle, 1961). In addition, carbachol probably acts directly at these postjunctional cholinergic receptors, as it does at the motor end plate of skeletal muscles (Koelle, 1970). It seems then, that the probable facilitatory mechanism of action for carbachol involves a decrease in the resting membrane potential which promotes an increase in excitability, presumably by promoting the entry of sodium into neurons.

Injection of crystalline carbachol compounds directly into the brain has become a popular technique subject to a number of criticisms because of obvious mechanical, osmotic, and alkaline effects. Several investigators have reported that microscopic examination of the histological material from experiments on carbachol stimulation of the hypothalamus, amygdala, thalamus, and midbrain reticular formation has not revealed evidence of tissue damage greater than that produced by the cannula implant itself (Grossman, 1963;

Miller, et al., 1964). The injection of various centrally active agents (such as serotonin, norepinephrine or gamma-aminobutyric acid) and control substances (sodium chloride, sodium nitrate, barium chloride, physostigmine, sucrose, posterior pituitary extract in crystalline form) into the basal amygdaloid complex has failed to duplicate the effects of carbachol stimulation (Grossman, 1963; MacLean, 1957a; Miller, 1965; Stevens, et al., 1961). Chemical specificity has been further indicated by a comparison of the pH and osmotic effects of the various substances mentioned above (Grossman, 1963).

Although the side effects of carbachol are difficult to assess and may not have been duplicated precisely by any of the control agents, such a wide range of pH and osmotic properties have been covered in control tests that it appears improbable that these factors should account for the observed results. Moreover, Belluzzi & Grossman (1969) have shown that in the rat, scopolamine, an antagonist of carbachol, re-establishes a one-way avoidance response after it had been selectively blocked by carbachol administration.

TABLE 1

Summary of data for cats injected with carbachol

Cat No.	PPC[1]	PAD[2]	Approximate Amount Delivered in μg (No. Injec.)	Seizure	Local Electrical Activity Changes	Ictal Behavioral Change	Chronic Behavioral Change
BL-2607	P		25 (1)	+	↑	Anger-Attack	Died in 4 days
BL-2654	W		25 (1)	+	↑	Fear-Defense	Died in 2 days
BL-2683*	P		10 (1)	+	↑	Anger-Attack	-
BM-2683	P		13 (1)	+	↑	-	Died in 2 hours
BM-2690	W		26 (2)	-	↑	Fear-Escape	Overdocile
BL-2716	W		25 (1)	-	-	-	Died in 4 days
BL-2723	P		50 (2)	-	↑	Fear-Escape	Overfearful
BL-2725	P		25 (1)	-	-	-	-
BL-2728*	P		10 (1)	+	↑	Anger-Attack	-
BM-2729	P		13 (1)	-	↑	Anger-Attack	Overdocile
BM-2736	P		26 (2)	+	↑	-	Overirritable
BM-2778	P		65 (5)	+	↑	Fear-Defense	Overdocile
BM-2850	P	↑	39 (3)	+	↑	-	-
BM-2878	W		13 (1)	+	↑	Unprovoked Rage	Died in 2 hours
BM-2902	P	↑	65 (5)	+	↑	Anger-Attack	Overirritable
BL-3014	W	↑	65 (5)	-	↑	-	-
BL-3044	P		26 (2)	+	↑	Unprovoked Rage	Died in 2 hours
BM-4014	P	↑	52 (4)	-	↑	-	Overirritable
BM-4015	P	↑	65 (5)	-	-	-	-
BM-4016	W	↑	26 (2)	+	↑	-	Overfearful
BM-4052	W	↑	65 (5)	-	↑	-	-

[1]Precarbachol Personality Classification
[2]Postcarbachol After-Discharge Threshold Level
P = Playful
W = Withdrew
BL = Basolateral Amygdala
BM = Basomedial Amygdala

* = Liquid Injection
↑ = Increased
+ = Present
- = No Change

TABLE 2

Comparison between mean after-discharge threshold levels in milliamperes for control cats, carbachol cats with convulsions, and carbachol cats without convulsions

	Weeks							
	1	2	3	4	5	6	7	8
Control Cats	2.5	2.2	2.3	2.55	-	-	-	-
Carbachol Cats with Generalized Seizures	2.05	2.4	3.7	4.1	3.9	6.1	7.3	15.0
Carbachol Cats with Focal Seizures	2.8	2.4	5.1	3.4	-	5.6	5.6	6.1
Carbachol Cats without Seizures	2.55	2.0	3.2	3.1	4.7	4.5	4.9	5.5

TABLE 3

Comparison between mean after-discharge threshold levels in milliamperes for carbachol cats with chronic behavioral alterations and carbachol cats without chronic behavioral alterations

	Weeks							
	1	2	3	4	5	6	7	8
Chronic Behavioral Alteration	2.2	2.4	4.2	4.1	3.9	6.1	7.3	12.2
No Behavioral Alteration	2.5	2.2	3.8	3.25	4.7	5.05	5.6	6.1

FIGURE LEGENDS

Abbreviations for all figures:

RA = right amygdala

RHT = right hypothalamus

RHC = right hippocampus

RS = right septum

RM = right motor

RO = right occipital

LA = left amygdala

LHT = left hypothalamus

LHC = left hippocampus

LS = left septum

LM = left motor

LO = left occipital

I = indifferent

The voltage calibration in all traces refers to the amygdala electrodes-

Figure 1. Ictal fear-escape behavior elicited from basomedial nucleus of the amygdala.

a. Control recording previous to chemical injection.

b. Tracing 10 minutes following carbachol implantation associated with quick searching movements of the eyes and head as the animal looked about in a state of apprehension.

c. Tracing 12 minutes after carbachol injection associated with the cat looking wildly about as if trying to seek an escape.

d. Tracing 15 minutes after carbachol injection associated with frightened look.

e. Tracing 20 minutes after carbachol injection associated with hypervigilence.

f. Tracing 30 minutes after carbachol following a focal motor seizure associated with the cat lying in bottom of recording box.

Calibration = 200 μV and 1 second

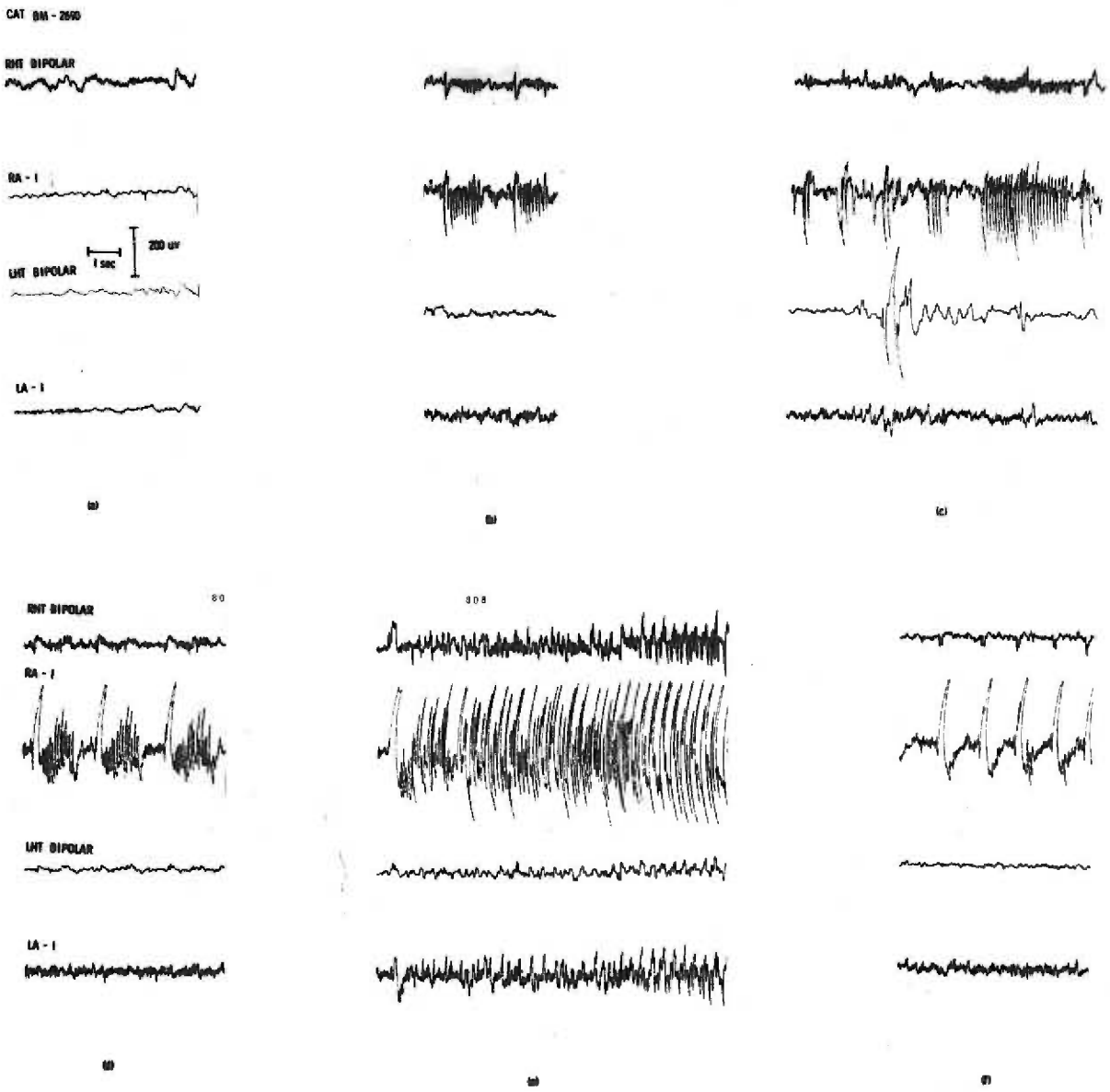

CAT BM - 2690
RHT BIPOLAR
RA - I
1 sec
200 uv
LHT BIPOLAR
LA - I
(a)
(b)
(c)
80
RHT BIPOLAR
RA - I
LHT BIPOLAR
LA - I
(d)
308
(e)
(f)

Figure 2. Temporal profile of electroencephalographic and averaged evoked response (AER) associated with ictal unprovoked rage behavior elicited from the basolateral amygdala nucleus of Cat BL-3044.

a. Normal tracing previous to carbachol stimulation.

b. Tracing and AER from left hypothalamus 5 minutes after carbachol stimulation.

c. Tracing and AER a few minutes later associated with cessation of all motor activity.

d. Tracing and AER 10 minutes after carbachol stimulation associated with pupillary dilatation, salivation, chewing movements.

e. Tracing and AER 15 minutes after carbachol stimulation associated with rhythmic blinking of left eye.

f. Tracing and AER a few minutes later associated with synchronous blinking of the eyes and escape behavior.

g. Tracing and AER 20 minutes postcarbachol associated with rigid stance.

h. Tracing and AER a few minutes later associated with avoidance of olfactory stimulation.

i. Tracing and AER 25 minutes after carbachol implantation associated with savage attack behavior.

j. Tracing and AER a few minutes later associated with cat lying exhausted on its side.

AER calibration = 50 mV and 25 msec; sweep = 250 msec. Same sweep in all subsequent graphs.

Calibration = 50 and 100 μV and 1 second

Enhancetron calibration = 50 mV and 25 milliseconds

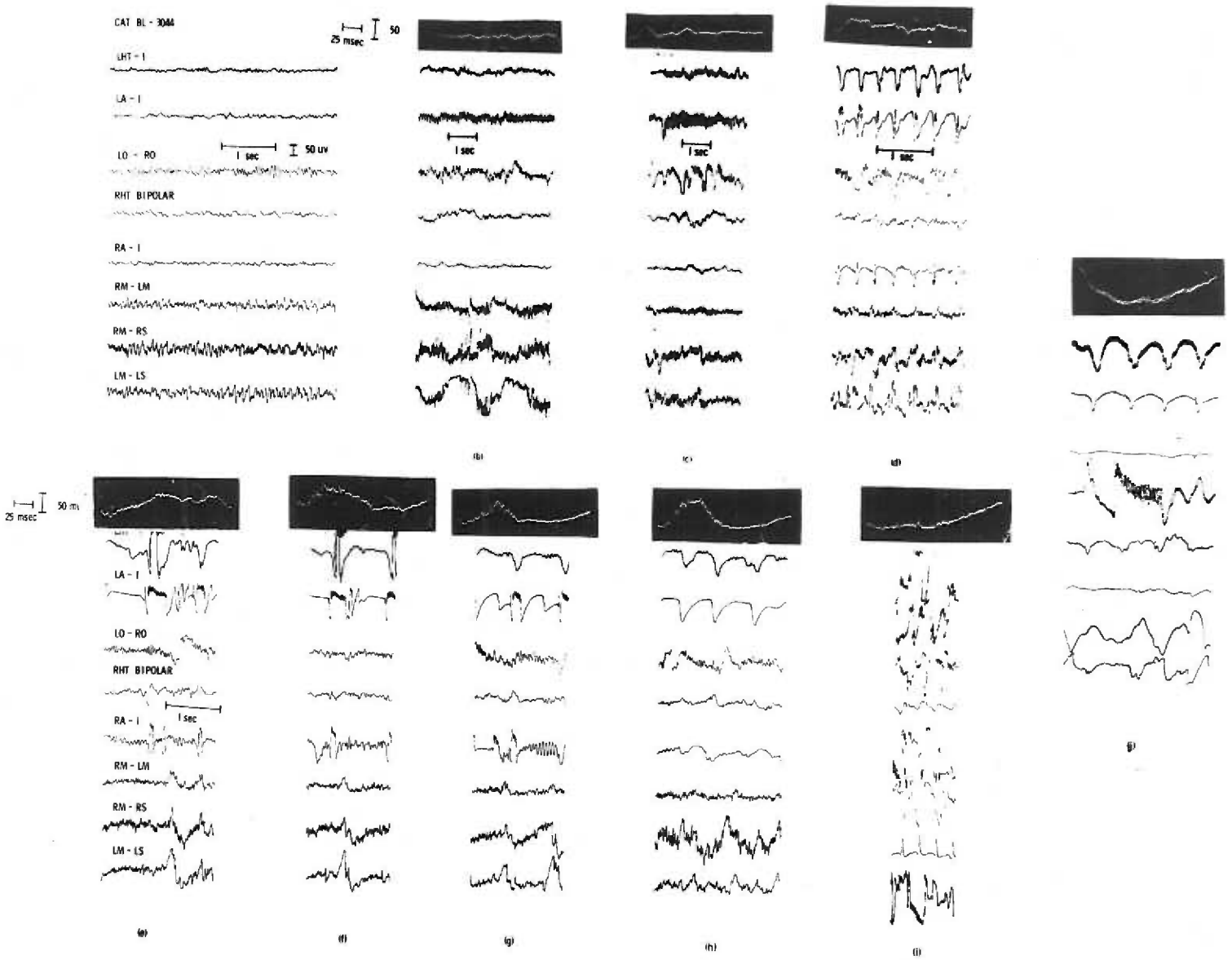

CAT BL - 3044
25 msec
50
LHT - I
LA - I
LO - RO
1 sec
50 uv
RHT BIPOLAR
RA - I
RM - LM
RM - RS
LM - LS
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)

Figure 3. Chronic overfearfulness elicited from basomedial nucleus of amygdala of Cat BM-4016.

a. Tracing of precarbachol stimulation response to alcohol olfactory stimulus.

b. Tracing of carbachol induced focal motor seizure.

c. Tracing of response to ether olfactory stimulus in midst of carbachol induced focal motor seizure.

d. Tracing 24 hours after carbachol stimulation.

e. Tracing of response to ether olfactory stimulus 24 hours after carbachol stimulation.

f. Tracing of response to auditory calling stimulus 24 hours after carbachol stimulation.

In the present record and all succeeding ones, the animal was presented with the stimulus at the arrow.

Calibration = 50 and 100 μV and 1 second

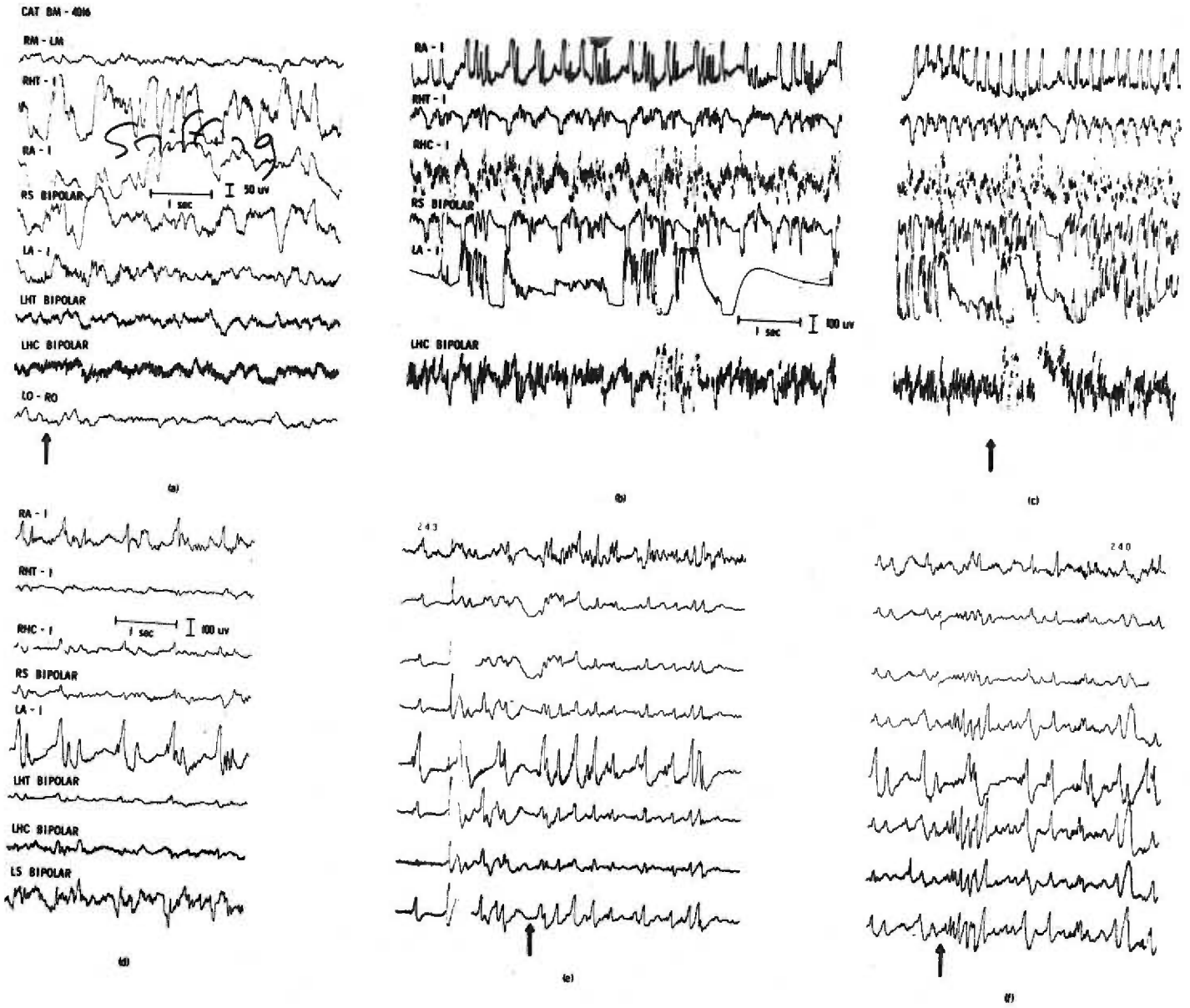

CAT BM - 4016
RM - LM
RHT - I
RA - I
RS BIPOLAR
1 sec
50 uv
LA - I
LHT BIPOLAR
LHC BIPOLAR
LO - RO
(a)
RA - I
RHT - I
RHC - I
RS BIPOLAR
LA - I
LHT BIPOLAR
LHC BIPOLAR
LS BIPOLAR
1 sec
100 uv
(d)
RA - I
RHT - I
RHC - I
RS BIPOLAR
LA - I
LHC BIPOLAR
1 sec
100 uv
(b)
243
(e)
(c)
240
(f)

Figure 4. Pathological reaction of obsessive interest shown by chronic overdocile cat to ether-soaked gauze associated with licking, rubbing over face, and nosing along floor 48 hours after the onset of carbachol induced amygdala spikes and 24 hours after recovery from generalized seizures.

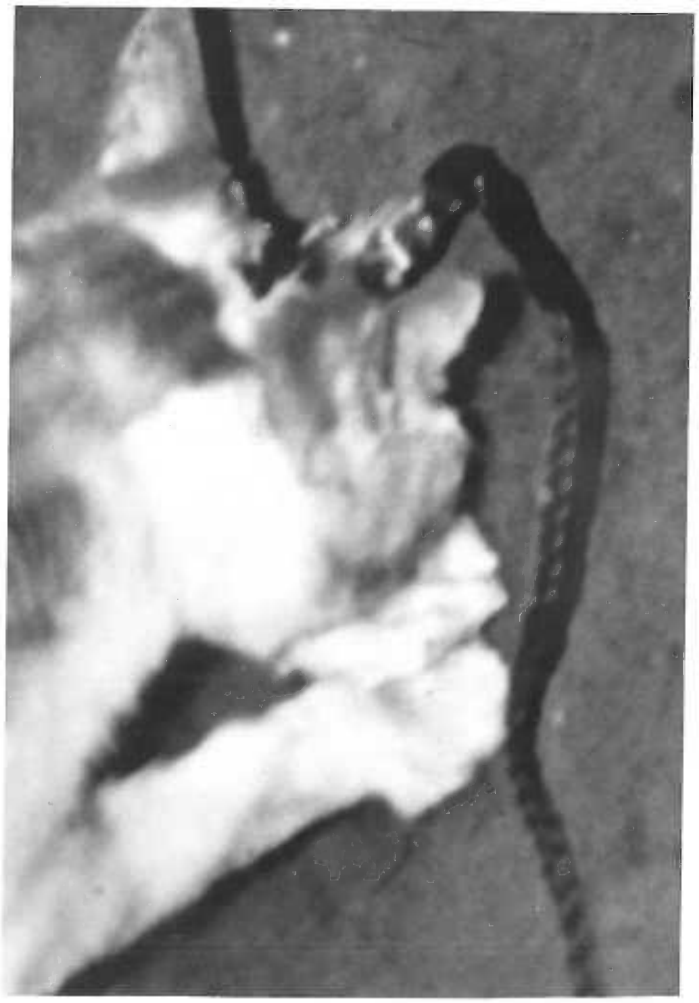


Figure 5. Ictal anger-attack behavior and chronic overdocility elicited from basomedial nucleus of amygdala of Cat BM-2902.

a. Control record previous to carbachol stimulation.

b. Response to noxious ether stimulus during control period.

c. Response to noxious ether stimulus 24 hours after generalized seizures.

d. Tracing 24 hours after carbachol stimulation associated with anger-attack behavior.

e. Tracing 24 hours after carbachol stimulation associated with rolling behavior.

f. Tracing 72 hours after carbachol stimulation.

g. Tracing 7 days after carbachol stimulation.

Calibration = 200 μV and 1 second

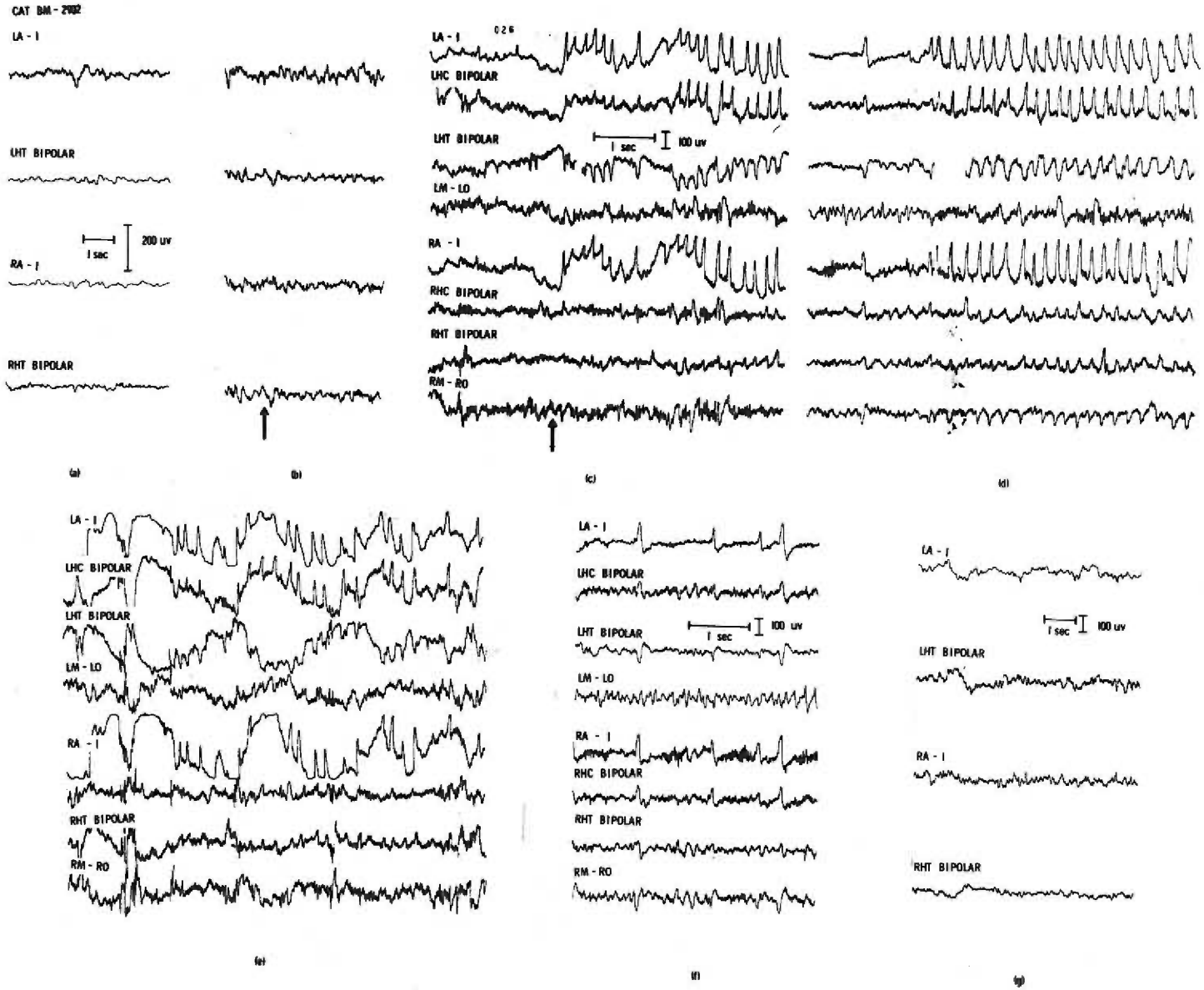

CAT BM - 2902
LA - I
LHT BIPOLAR
1 sec
200 uv
RA - I
RHT BIPOLAR
(a)
(b)
LA - I
LHC BIPOLAR
LHT BIPOLAR
1 sec
100 uv
LM - LO
RA - I
RHC BIPOLAR
RHT BIPOLAR
RM - RO
(c)
(d)
LA - I
LHC BIPOLAR
LHT BIPOLAR
LM - LO
RA - I
RHC BIPOLAR
RHT BIPOLAR
RM - RO
(e)
LA - I
LHC BIPOLAR
LHT BIPOLAR
1 sec
100 uv
LM - LO
RA - I
RHC BIPOLAR
RHT BIPOLAR
RM - RO
(f)
LA - I
1 sec
100 uv
LHT BIPOLAR
RA - I
RHT BIPOLAR
(g)

Figure 6. Ictal anger-attack behavior elicited from basomedial nucleus of the amygdala.

a. Salivation, licking and pupillary dilatation during the initial onset of carbachol induced amygdala spikes.

b. Provoked threat pattern elicited 20 minutes after onset of carbachol induced amygdala spikes associated with pupillary dilatation, retraction of ears, hissing, growling and snarling.

(a)

(b)

Figure 7. Ictal fear-escape behavior elicited from medial nucleus of the amygdala.

a. Initial reaction to onset of carbachol induced amygdala spike activity associated with salivation, pupillary dilatation, and staring.

b. Twenty minutes after onset of carbachol spikes cat suddenly sprang back as if afraid of something in corner of recording box.

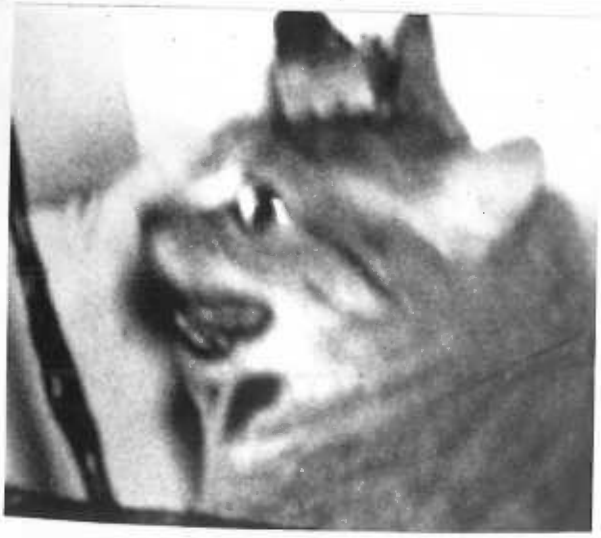
c. Twenty-five minutes after onset of carbachol spikes cat displays hypervigilant behavior as it stares intently at corner of recording box while salivating profusely.

d. Thirty minutes after onset of carbachol spikes cat suddenly starts backing away from corner of box associated with profuse salivation, pupillary dilatation, piloerection, and terrified look.

(a)

(b)

(c)



(d)

Figure 8. Temporal profile of electroencephalographic and averaged evoked response activity of Cat BM-2850 associated with ictal focal motor seizures.

a. Normal tracing of spontaneous electrical activity 10 days after implantation during control period immediately preceding chemical stimulation.

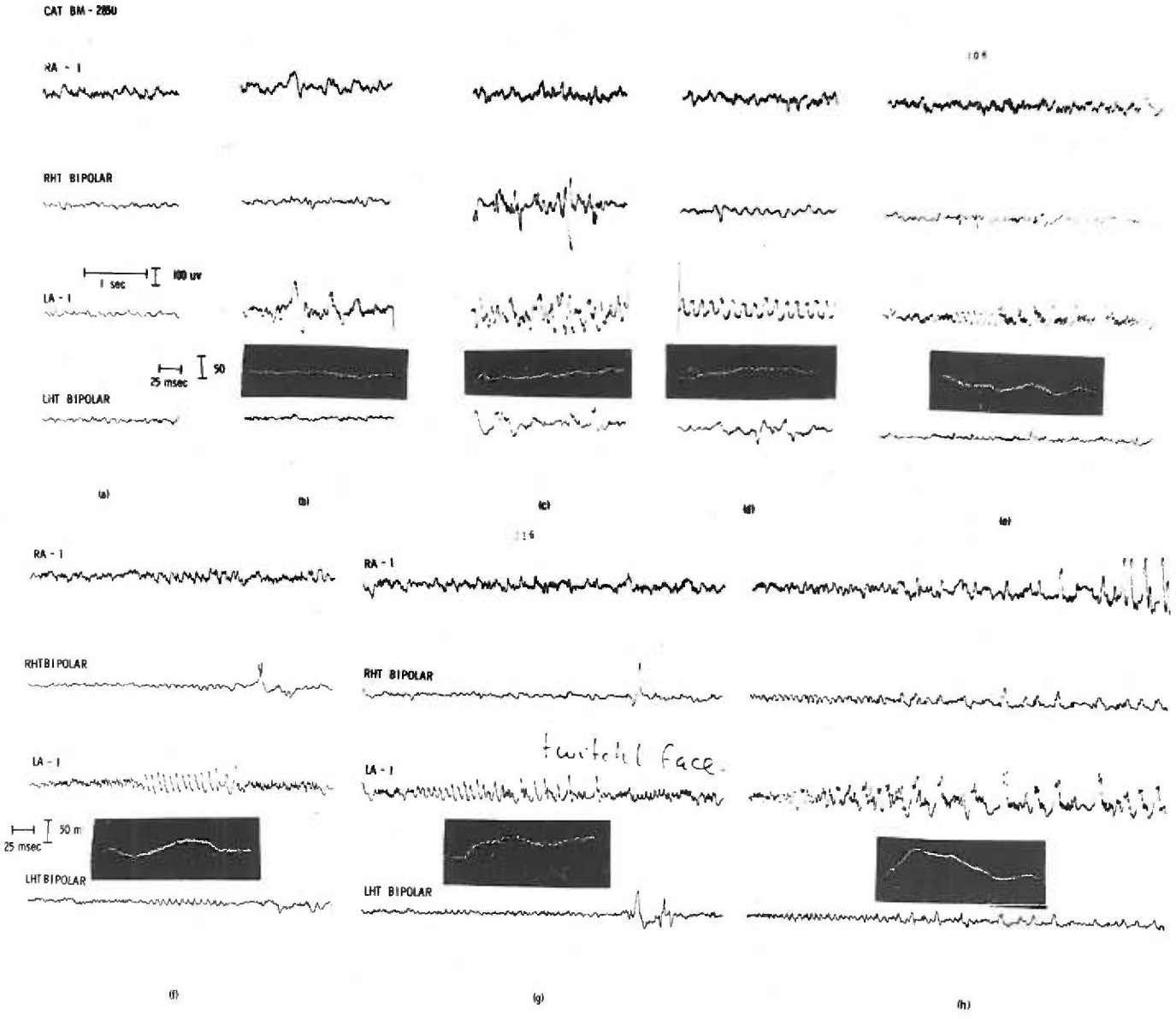
b. Tracing and AER few minutes after bilateral administration of carbachol associated with sniffing catnip mouse.

c. Tracing and AER 5 minutes after carbachol stimulation associated with sniffing floor of cage.

d. Tracing and AER 10 minutes after carbachol stimulation associated with sniffing at the air.

e. Tracing and AER 15 minutes after carbachol stimulation associated with muscular twitching of the left face.

f. Tracing and AER 17 minutes after carbachol stimulation associated with left facial spasms and rhythmic blinking of both eyes.


CAT BM - 285U
RA - 1
RHT BIPOLAR
1 sec
100 uv
LA - 1
25 msec
50
LHT BIPOLAR
(a)
(b)
(c)
(d)
(e)
RA - 1
RHTBIPOLAR
LA - 1
50 m
25 msec
LHTBIPOLAR
(f)
RA - 1
RHT BIPOLAR
twitch face.
LA - 1
LHT BIPOLAR
(g)
(h)

g. Tracing and AER 25 minutes after carbachol stimulation associated with twitching of entire left side.

h. Tracing and AER 30 minutes after chemical stimulation associated with visual searching behavior.

i. Tracing and AER 35 minutes after carbachol stimulation associated with pupillary dilatation, profuse salavation and focal motor seizure.

j. Two hours following onset of focal motor seizures associated with slowing of background activity.

k. Response to pleasant milk olfactory stimulus 2 hours after carbachol stimulation.

l. Response to photic stimulation 2 hours after carbachol stimulation.

m. Response to petting 2 hours after carbachol stimulation.

n. Response to noxious ether olfactory stimulus 2 hours after carbachol stimulation.

o. Twenty-four hours after carbachol stimulation.

Calibration = 50 and 100 μV and 1 second

Enhancetron calibration = 50 mV and 25 milliseconds

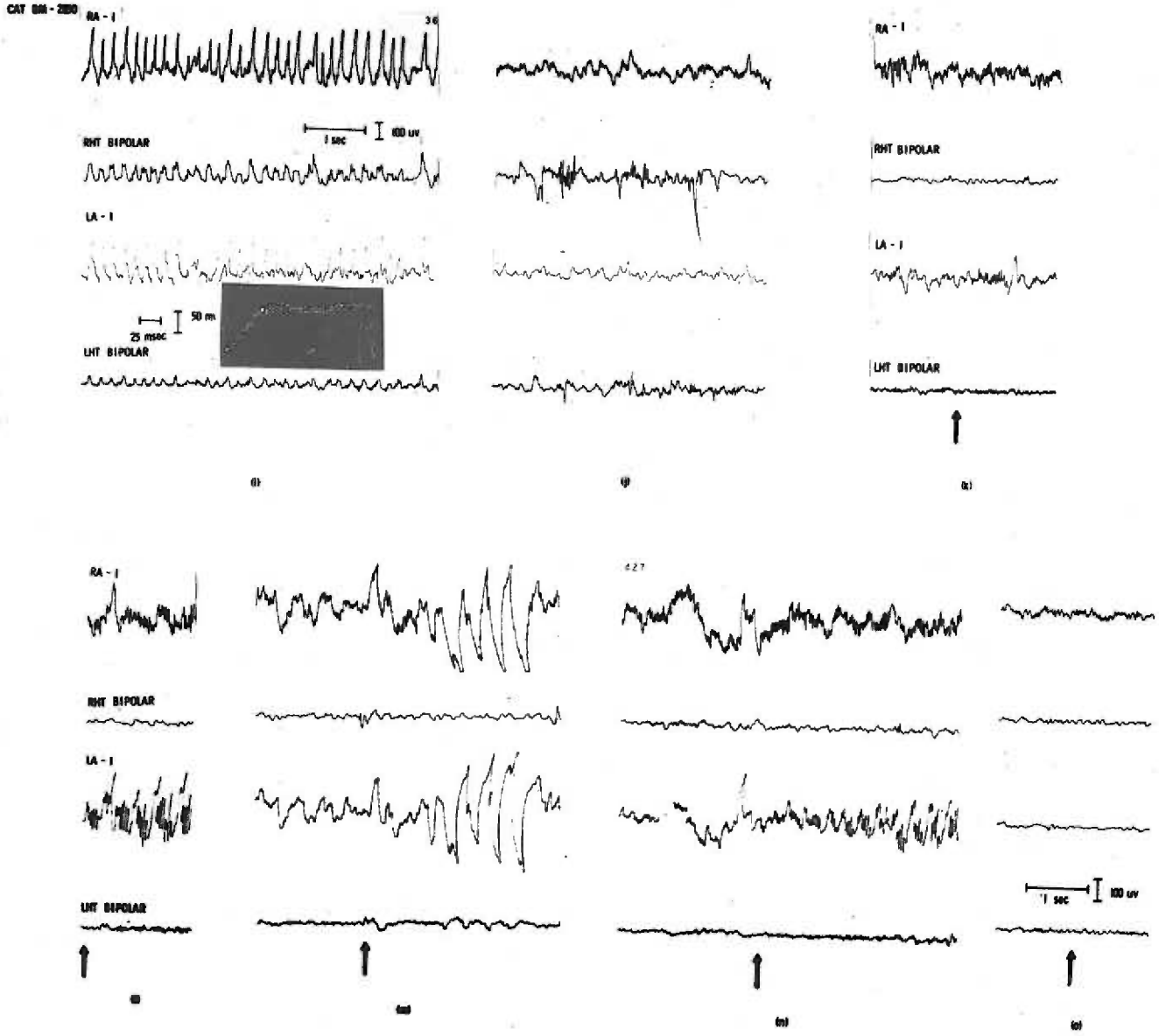

RA - I
RHT BIPOLAR
LA - I
LHT BIPOLAR
1 sec
100 uv
50 m
25 msec

Figure 9. Ictal electroencephalographic response without behavior elicited from basomedial nucleus of amygdala of Cat BM-4014.

a. Control recording a few minutes before carbachol administration.

b. Tracing during midst of carbachol-induced change of electrical record associated with no behavioral correlates.

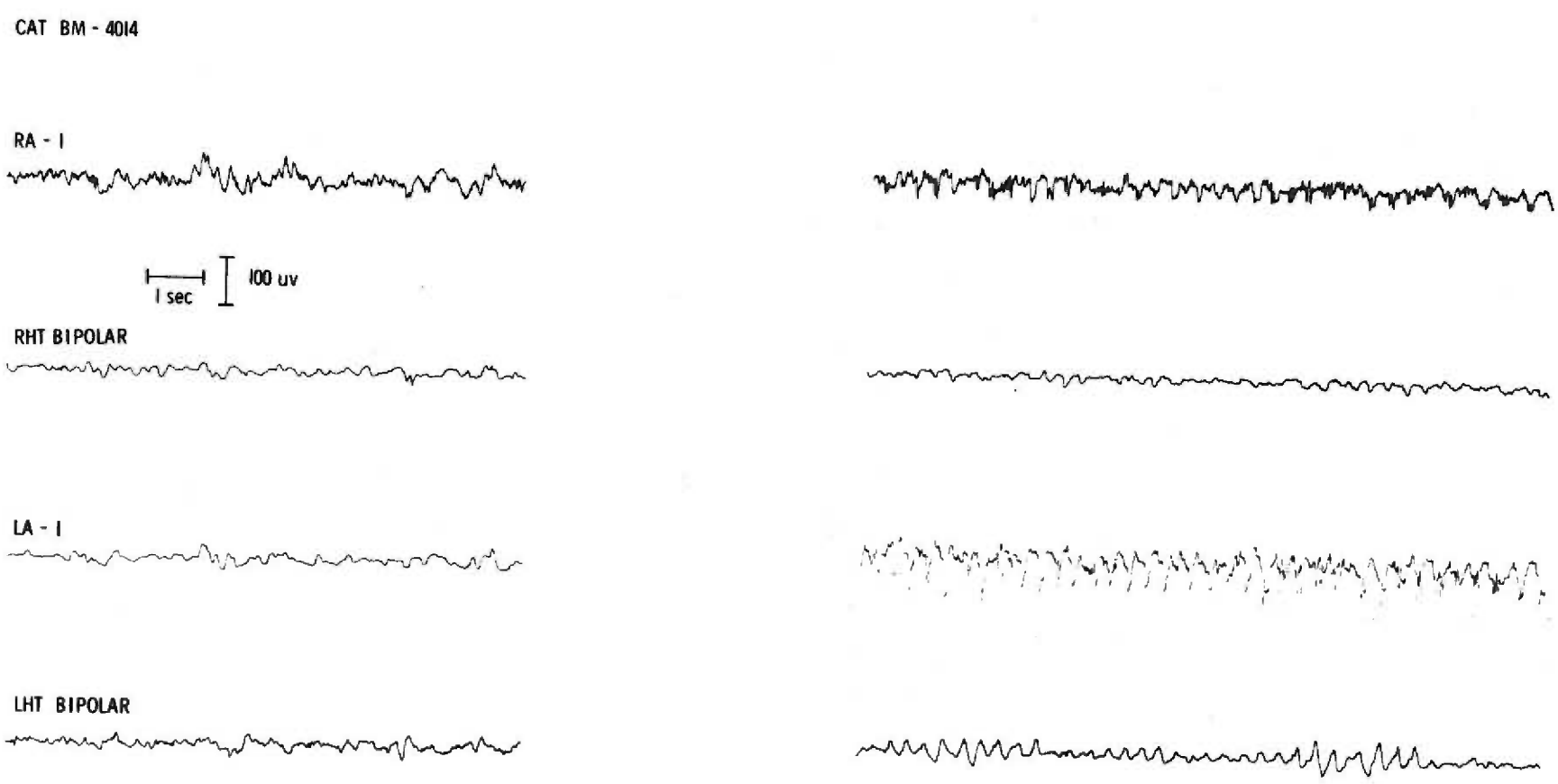

CAT BM - 4014
RA - I
1 sec
100 uv
RHT BIPOLAR
LA - I
LHT BIPOLAR
(a)
(b)

Figure 10. Ictal fear-defense behavior and chronic overdocility elicited from basomedial nucleus of the amygdala of cat BM-2778.

a. Tracing recorded during carbachol induced focal motor discharges associated with fear-defense behavior.

b. Growling defense reaction.

c. Tracing 24 hours after carbachol implantation stimulation.

d. Tracing 48 hours after carbachol implantation stimulation.

e. Response to pleasant olfactory tuna fish stimulus 48 hours after carbachol stimulation.

f. Response to noxious ether stimulus 48 hours after carbachol stimulation.

g. Tracing 10 days after carbachol stimulation.

h. Response to noxious ether stimulus 10 days after carbachol stimulation.

Calibration = 50 and 100 μV and 1 second

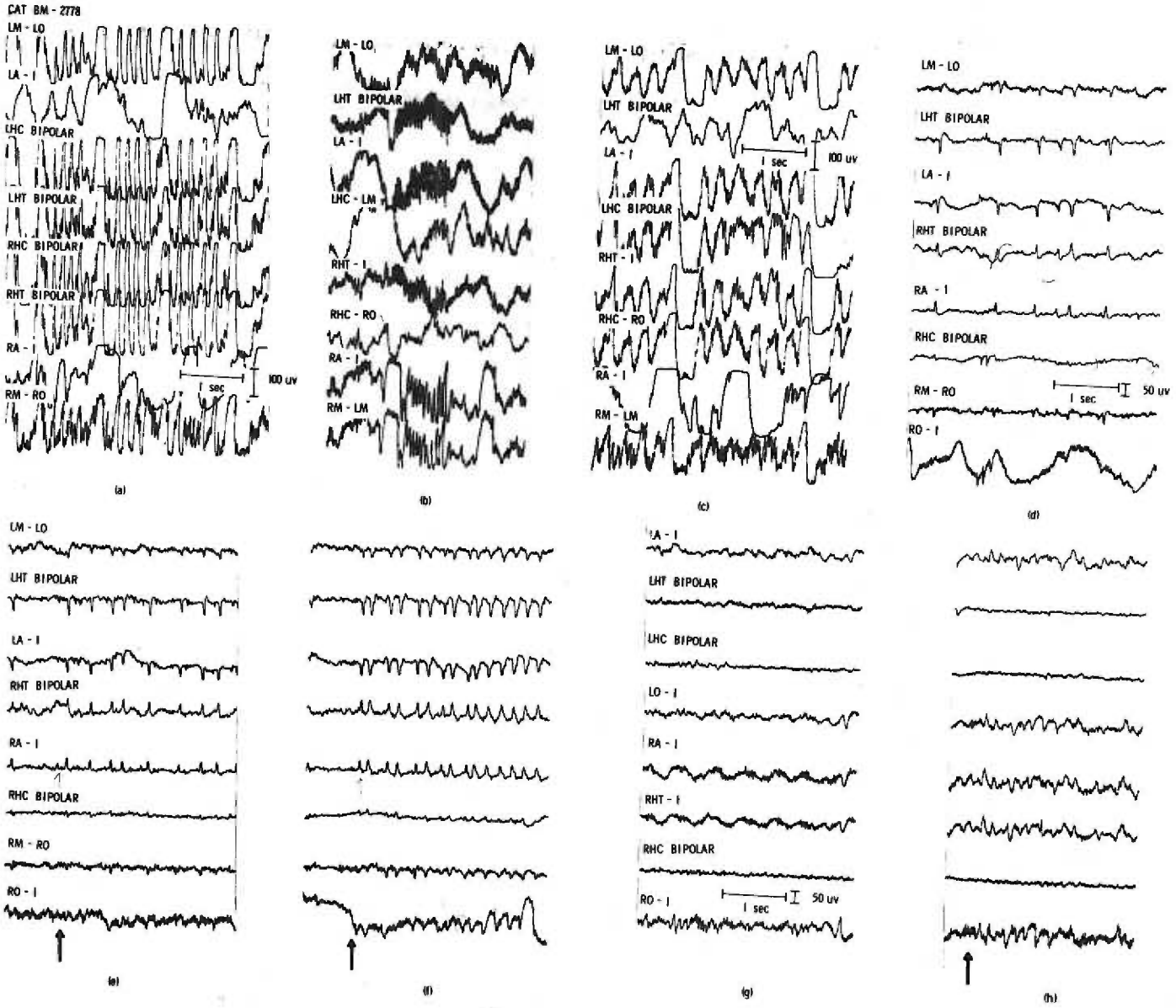
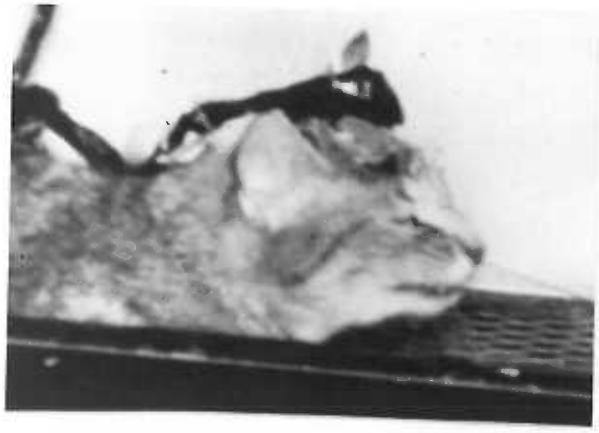

CAT BM - 2778
LM - LO
LA - I
LHC BIPOLAR
LHT BIPOLAR
RHC BIPOLAR
RHT BIPOLAR
RA - I
RM - RO
1 sec
100 uv
(a)
LM - LO
LHT BIPOLAR
LA - I
LHC - LM
RHT - I
RHC - RO
RA - I
RM - LM
(b)
LM - LO
LHT BIPOLAR
LA - I
LHC BIPOLAR
RHT - I
RHC - RO
RA - I
RM - LM
1 sec
100 uv
(c)
LM - LO
LHT BIPOLAR
LA - I
RHT BIPOLAR
RA - I
RHC BIPOLAR
RM - RO
RO - I
1 sec
50 uv
(d)
LM - LO
LHT BIPOLAR
LA - I
RHT BIPOLAR
RA - I
RHC BIPOLAR
RM - RO
RO - I
(e)
(f)
LA - I
LHT BIPOLAR
LHC BIPOLAR
LO - I
RA - I
RHT - I
RHC BIPOLAR
RO - I
1 sec
50 uv
(g)
(h)

Figure 11. Behavioral responses of waking cat to electrical stimulation of basal medial nucleus of amygdala.

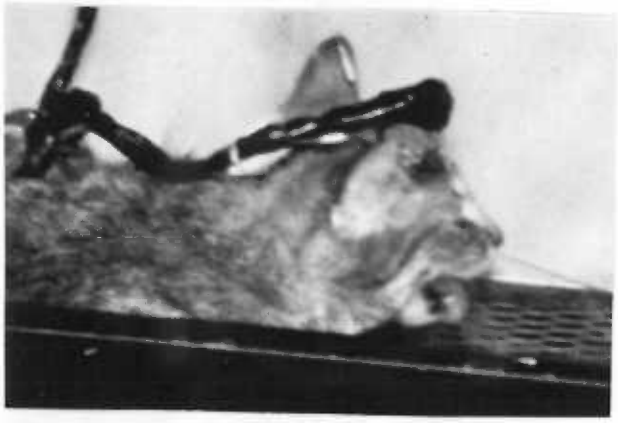
a. Low intensity stimulation elicits stimulus bound rhythmic blinking.

b. Moderate intensity stimulation elicits stimulus locked rhythmic blinking and chewing.

c. High intensity stimulation elicits rhythmic blinking, chewing, movements of the nostrils, facial muscle contractions, and gagging.



(a)



(b)

(c)

Figure 12. Temporal profile of electroencephalographic and AER activity of basomedial nucleus of amygdala of cat BM-4014 during electrical stimulation procedure.

a. Control recording previous to electrical stimulation.

b. Response to 0.2 mA current.

c. Response to 0.4 mA current.

d. Response to 0.6 mA current.

e. Response to 0.8 mA current.

f. Response to 1.0 mA current

g. Response to 1.2 mA current.

h. Response to 1.4 mA current.

i. Response to 1.6 mA current.

j. Response to 1.8 mA current.

k. Tracing of after-discharge elicited from basomedial amygdala.

Calibration = 100 μV and 1 second

Enhancetron calibration = 50 and 100 mV and 25 milliseconds

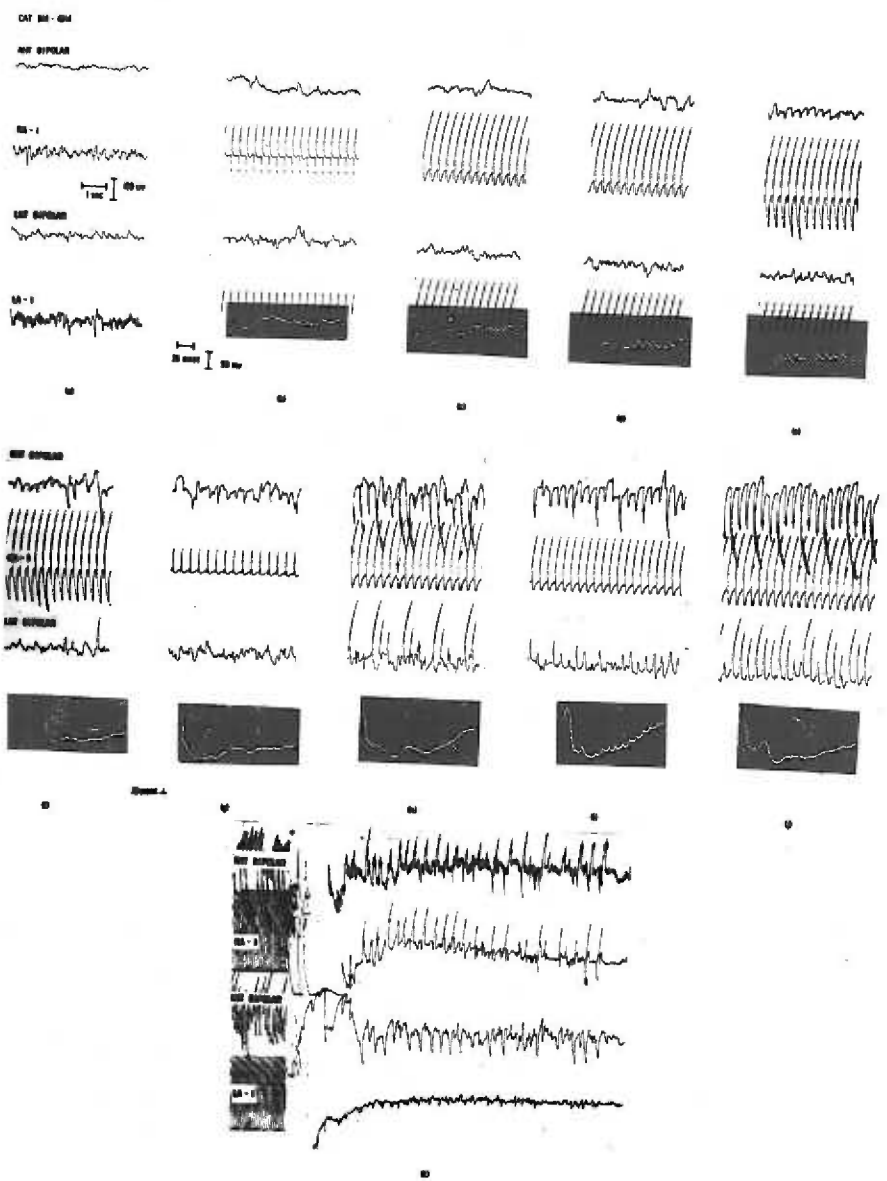


Figure 13. Precarbachol after-discharge threshold levels of experimental and control animals.

o = Basolateral Amygdala Animals

● = Basomedial Amygdala Animals

▫ = Control Animals

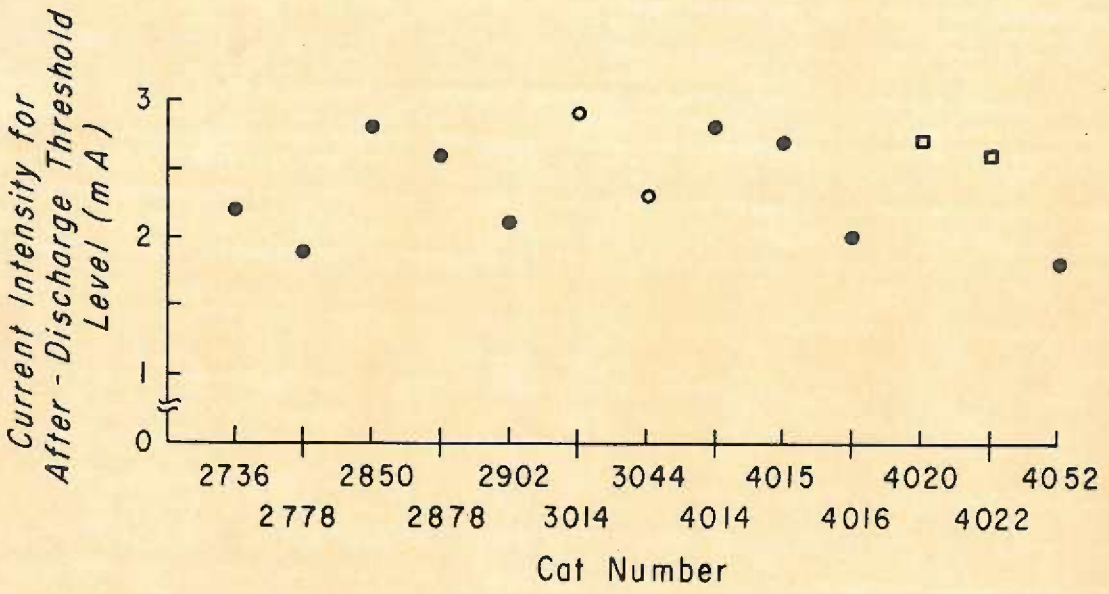

Current Intensity for After-Discharge Threshold Level (mA)
3
2
1
0
2736
2778
2850
2878
2902
3014
3044
4014
4015
4016
4020
4022
4052
Cat Number

Figure 14. After-discharge threshold level of control animals for four consecutive weeks.

o = Cat BM-4020

● = Cat BM-4022

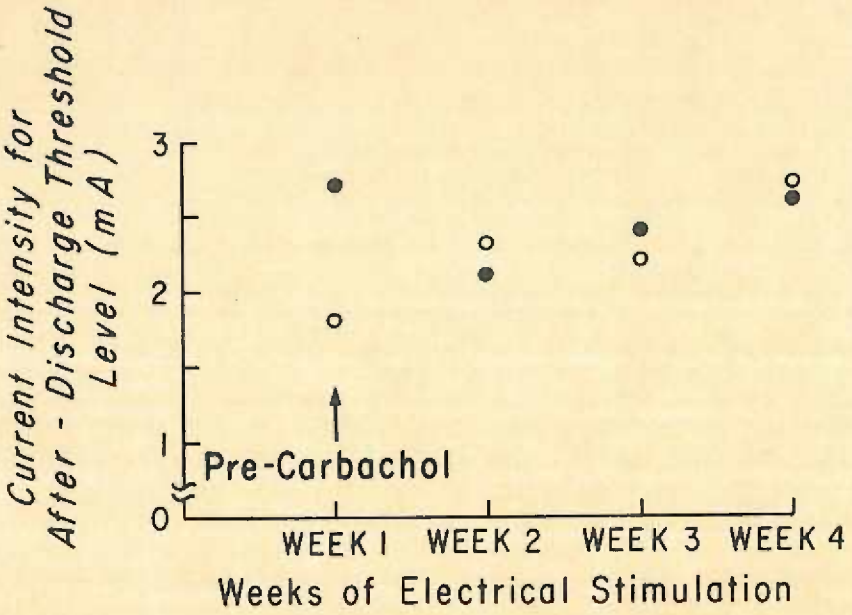

Current Intensity for After - Discharge Threshold Level (mA)
3
2
1
0
Pre-Carbachol
WEEK 1
WEEK 2
WEEK 3
WEEK 4
Weeks of Electrical Stimulation

Figure 15. Square wave of electrical stimulus signal channel which triggered 250 msecond sweep of Enhancetron on upper channel and electrical pulse artifact as recorded in ventromedial hypothalamus on lower channel.

Calibration: 1 volt and 10 mseconds.

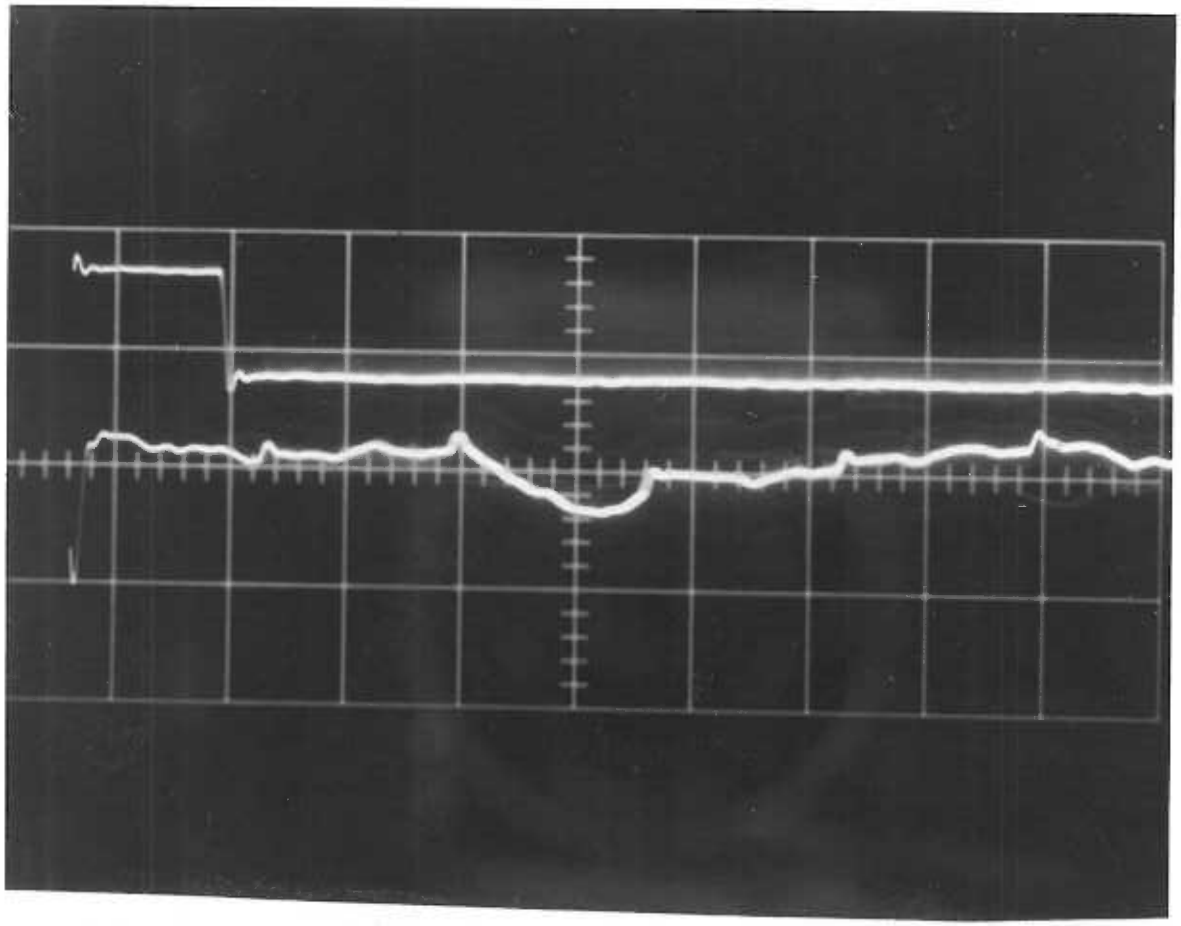

10 msec
1 V

Figure 16. Cross section of cat brain showing site and extent of cannula tracts in the basomedial nucleus of the amygdala. Luxol-Fast-Blue-Periodic-Acid-Schiff-Hematoxylin-stained section, magnified X 5.

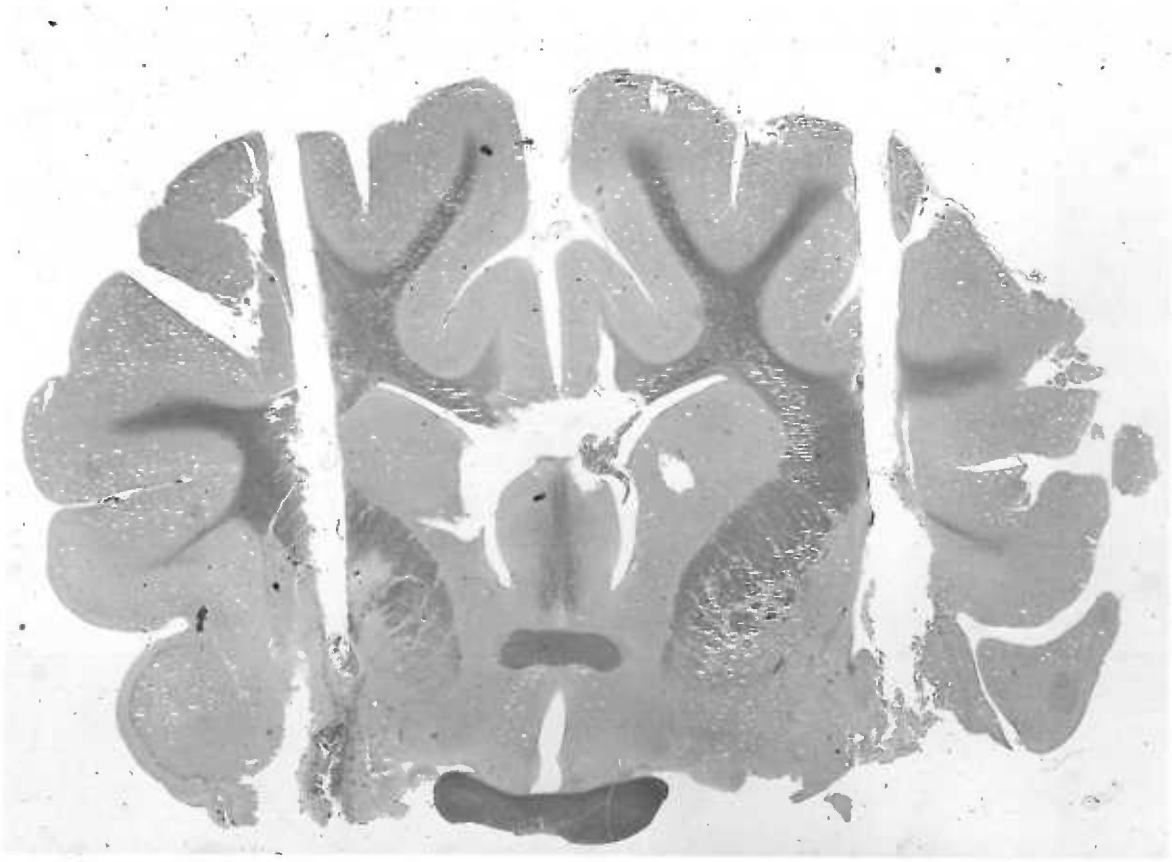


Figure 17. Typical gliosis around tip of cannula in control cat. The tips in the basomedial nucleus of the amygdala. Luxol-Fast-Blue-Periodic-Acid-Schiff-Hematoxylin-stained section, magnified X 75.

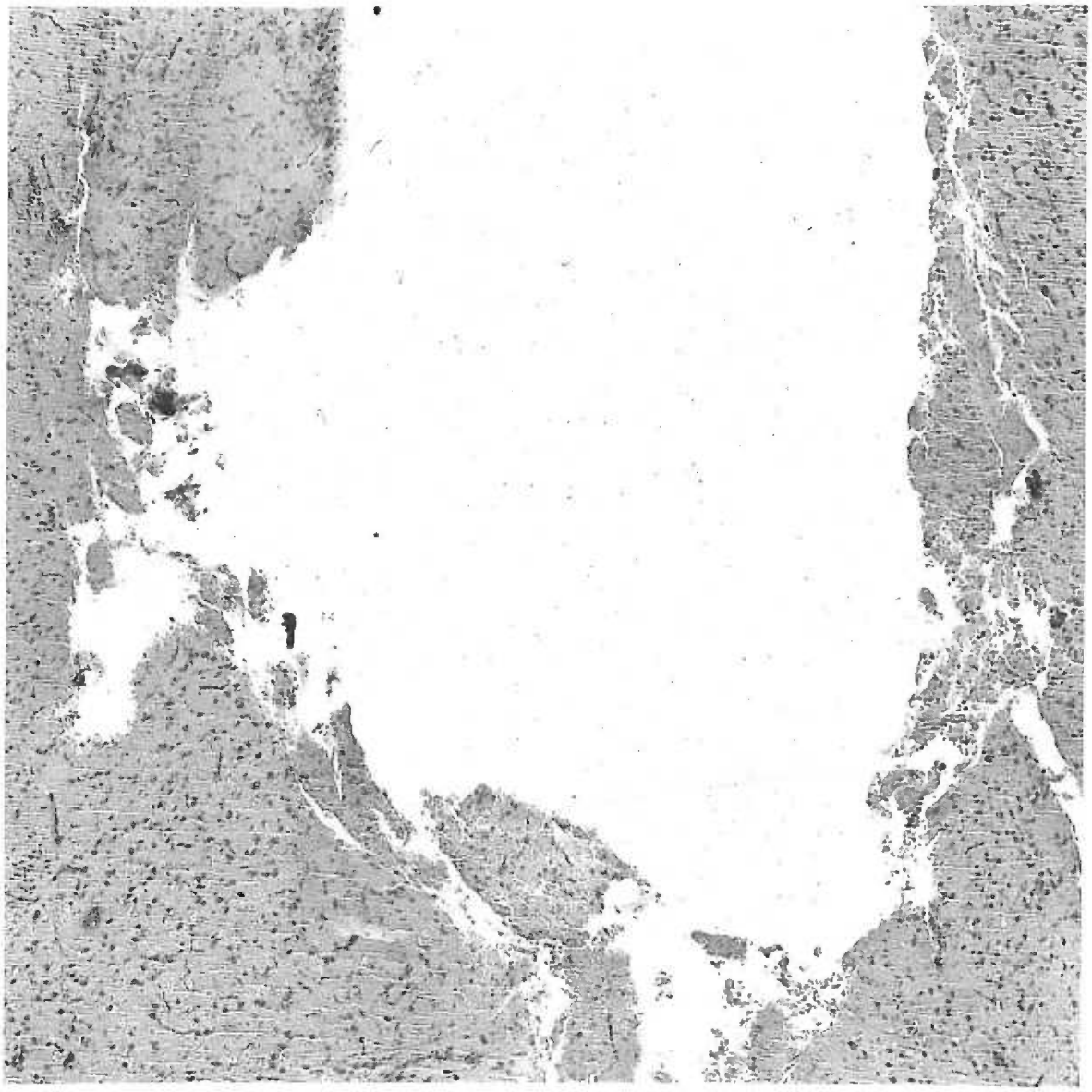


Figure 18. Typical tissue destruction extending away from cannula tip in carbachol-injected cat. The tip, in the lower right corner, is in the basomedial nucleus of the amygdala. Luxol-Fast-Blue-Periodic-Acid-Schiff-Hematoxlyin-stained section, magnified X 75.