BEHAVIORAL AND EEG PATTERNS IN THE CAT COINCIDENT WITH SYSTEMIC AND INTRACRANIAL STIMULATION WITH d-AMPHETAMINE SULFATE DURING A VISUAL DISCRIMINATION TASK

A Dissertation

Presented to

the Faculty of the Department of Psychology

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Richard L. Miller

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ABSTRACT

The behavioral and electroencephalographic (EEG) effects of d-amphetamine sulfate injection into the basolateral amygdala, posterior hypothalamus, ventral hippocampus, and medial thalamic area were compared with a saline control injection, and a central administration of amphetamine delivered via the interperitoneal cavity during a behavioral task. The order of drug administration to the respective areas in each of six adult male cats was balanced in a latin-square design. The experimental task consisted of a 50 trial complex go-no-go visual discrimination task. Each trial consisted of a 10 second reinforcement period (SD) cued by a 4 c/sec. flashing light. Bar pressing responses made during the SD period were reinforced with milk. The intertrial period was a 30 second variable interval schedule during which no reinforcement could be obtained. Quantitative measures obtained on all subject consisted of total responses, reinforced responses (SD_R), and intertrial interval responses (ITI_R). Daily performances were evaluated on the basis of a ratio of intertrial interval to reinforced responses (ITI_R / SD_R). In addition EEG measures were obtained from electrodes implanted in various brain loci and permanently stored on tape. EEG records were obtained on the stimulation day, while operant levels were recorded 24 and 48 hours following stimulation as well.

Comparisons were made between baseline, saline, and experimental conditions for the operant behavioral results as

follows:

(1) Amphetamine stimulation in the basolateral amygdaloid nuclei was characterized by significant facilitation of performance at 24 and 48 hours following stimulation.

(2) Posterior hypothalamic stimulation resulted in significant increases in performance at 24 and 48 hours, although not of the same magnitude as the amygdala results.

(3) Hippocampal and thalamic stimulations with amphetamine were found to be non-significant in their effects on operant responses.

(4) Central stimulation with amphetamine produced operant levels which trended toward facilitation at 24 hours and reached significance at 48 hours.

(5) Saline administrations were found to be non-effective in influencing operant responding, differences between the saline control and baseline being non-significant.

Analyses of five frequency bands in the EEG arousal spectrum were performed, concentrating solely on the amygdaloid stimulation days, with examination of brain loci confined to the lateral geniculate and visual cortex, the primary projection pathways for the visual stimulus. The principle results were as follows:

(1) Amphetamine stimulation in the amygdala produced shifts in the frequency bands resulting in a dominance of power gains coincident with the photic stimulus pulsations in the band characterized by a center frequency of 40 c/sec.

This result was seen in both the visual cortex and lateral geniculate.

(2) Crosspower functions which measured the coherence of power in a frequency band for the two areas was calculated with the result that again the 40 c/sec. band was prevalent.

The results of these findings were related to the psychopharmacological properties of amphetamine and its actions on norepinephrine, as well as neuroanatomical structures in the brain possessing high concentrations of norepinephrine. The amygdala and hypothalamus were implicated as unusually active in this context. Further implications were drawn regarding the prevalence of 40 c/sec. electrical activity as an electrophysiological correlate of learning.

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CHAPTER I

INTRODUCTION

The biochemistry of the central nervous system has played a significant role in the development of the area of psychopharmacology as a relevant scientific discipline for the study of behavior. As the mechanisms of drug actions become more clearly understood, the relationships between biochemical and physiological processes in the central nervous system become more elucidated.

The central nervous system has evolved as a specialized system of circuitry of which the primary function is the processing of sensory and motor information. In the areas and systems in which neurophysiologists have been able to map circuitry for the controls for basic drives such as eating, drinking, or sexual and maternal behavior, a central chemical code has been discovered. (Stellar, 1954; Miller, 1965)

However, chemical activity and reactivity in the brain is extremely complex as is demonstrated by the following examples: (1) It has been shown that the same hormone placed in different areas of the hypothalamus can produce distinctly male or female types of behavior, depending upon the site of

stimulation. On the other hand, (2) when different chemical agents which either mimic or block the actions of brain neurohumors are applied to that same brain area by micro-cannula, selective eating or drinking is elicited, dependent upon whether the neurohumor is adrenergic or cholinergic in nature (Fisher, 1964).

Generally a neurohumor must meet the following criteria to be considered a transmitter: (1) it must mimic the action of a neural action potential; (2) its effectiveness must change as a function of the same chemical or neural manipulations which are known to alter neural excitation; (3) it must occur naturally, preferably in non-random pools or distributions; and (4) a naturally occurring antagonist must inactivate the neurohumor of interest as rapidly as is required. This may necessitate inactivation of as many as 2000 neural potentials per second (Grossman, 1967).

Transmitters are hypothesized to be selectively excitatory or inhibitory in their action on the post-synaptic membrane. Excitors depolarize the post-synaptic membrane, thereby lowering the potential differences between the interior and exterior of the cell wall. This action is considered as an excitatory post-synaptic potential (EPSP). Inhibitors produce an inhibitory post-synaptic potential (IPSP) by hyperpolarization of the post-synaptic membrane, thereby lowering the excitability of the post-synaptic neural level. The magnitude of the post-synaptic effect is considered to

be a function of the quantity of neurohumor acting upon the post-synaptic membrane.

Synaptic conduction in the central nervous system can be broadly classified as bound within two categories. Nerve fibers which activate effector cells innervated by fibers of the craniosacral and somatic efferent nerves are called parasympathetic pathways and are considered cholinergic in nature (Thompson and Schuster, 1968). Pathways in the sympathetic system are adrenergic in nature and produce a variety of physiological responses when stimulated: cutaneous vasoconstriction, skeletal muscle vasodilatation, mydriasis, pilomotor contraction, etc.(Thompson and Schuster, 1968).

The free-occurring neurohumors in the central nervous system felt most closely to satisfy the adrenergic and chorinergic systems are norephinephrine and acetylcholine respectively. Other substances have been identified which produce sufficiently marked effects on the central nervous system as to be suspect as acting upon the transmission characteristics of certain systems of the brain. The most promising of these suspected transmitters is 5-hydroxytryptamine (serotonin) which appears to be selectively active in several parts of the brain. Gamma-aminobutyric acid (GABA) also appears likely as an inhibitory transmitter in that it selectively inactivates post-synaptic membranes.

Acetylcholine, as the principle cholinergic agent is effective in low concentrations at receptor sites, but is

rapidly destroyed by its natural antagonist, the enzyme, acetylcholine esterase. For this reason, only small amounts of the drug can reach receptor sites and, as such, acetylcholine is very difficult to deal with in in vivo experiments.

However, structures innervated by cholinergic nerves can be stimulated by two additional parasympathetic agents other than acetylcholine, or a structurally-similar compound. Cholinesterase inhibitors, such as physostigmine, neostigmine, and diisopropylfluorophospate (DFP) protect acetylcholine from hydrolysis and intensify and perpetuate the excitatory effect upon neural tissue (Grossman, 1967). The other class is the naturally occurring alkaloids, such as pilocarpine, arecoline, and muscarine. These chemicals produce a dual effect, first mimicing acetylcholine stimulation fr a short duration (depolarization), then inhibits (hyperpolarizes) the activity of cells in the antonomic ganglion and skeletal muscles (Thompson and Schuster, 1968).

Early work of Otto Loewi, while demonstrating that a neurohumor (acetylcholine) was released with parasympathetic stimulation in an organism, also encountered a neurohumor released with sympathetic innervation which produced opposite effects on the cardiac muscle than did acetylcholine. Loewi's "accelerator" substance turned out to be an adrenergic transmitter similar in structure to epinephrine and most closely resembeling norepinephrine.

It is known today that the substance is norepinephrine.

Pharmacologically, agents of a sympathomimetic class are structurally related to the natural prototype, epinephrine. Ephinephrine(adrenalin or suprarenin) and levarterenol (noradrenaline or norepinephrine) are naturally occurring substances which are involved in mediating adrenergic nueroeffector transmission (Thompson and Schuster, 1968).

The structure of the effector cell sensitive to free occurring adrenergic agents is still not clearly identified. Ephinephrine may serve as a stimulant to some cells innervated by an adrenergic nerve, while other cells, obviously activated by an adrenergic nerve, are inhibited by epinephrine. It is apparent, then, that a more complex system exists within the adrenergic receptors than within the cholinergic receptors. A hypothesis currently favored is that two types of adrenergic receptors exist, one excitatory(α-adrenergic) and the other inhibitory (Moran, 1966). That mode of receptor concerned with excitatory responses is primarily sensitive to epinephrine to a degree of two to ten times the magnitude of levarterenol.

The adrenergic receptor associated with inhibitory responses (β-adrenergic) is maximally sensitive to isopropylarterenol.

Epinephrine and levarterenol are destroyed by two antagonistic enzymes. One is monoamine oxidase(Ahlquist, 1958), which brings about oxidative deamination; the other, catechol-o-methyltransferase, effects o-methylation of the benzene ring of the compound. One means of retarding the deamination

is to block the action of monoamine oxidase(MAO) by a member of a second major group of sympathomimetics, the amphetamines.

Amphetamine, though related in structure to epinephrine, differs from it by possessing a high resistance to enzymatic destruction of its ring. Amphetamine exists in two pure forms and one racemic mixture. The dextrorotatory form (dexidrene) is the strongest of the three, having approximately twice the potentcy of the racemic form(amphetamine). The least effective form is the levoratatory structure(Benzedrine).

Amphetamine produces a profound effect on the central nervous system, where it accelerates and desynchronizes the electroencephalogram(Cahn and Herold, 1968; Knoll, 1966).

Administration of amphetamine has been reported to have a wide spectrum of effects on behavior. Maikel et al.(1969) has shown that minimum brain levels of d-amphetamine required to produce significant fluctuations in rat performances vary widely with the nature of the behavioral task. The most sensitive behavioral test is Sidman avoidance procedures which shows significant performance change with doses of d-amphetamine as low as 0.38 $\mu g/g$ body weight.

A large number of recent studies have suggested that brain norephinephrine in strongly involved in the mechanisms whereby amphetamine produces stimulation of the central nervous system(Hanson, 1966; 1967; Cox et al., 1968). The details of these and other relevant areas are considered in depth in Chapter II.

The ability of amphetamine to preserve and provoke the adrenergic action of its transmitters provided an incentive for the present research design.

Statement of the Problem

The general objectives of the experiments were: (1) to explore the effects of amphetamine within the cranium in selected subcortical structures, and (2) examine differences resulting from extracranial administration(interperitoneal injection) as compared to effects of intracranial stimulation.

These effects were examined at three levels: (1) general behavioral effects; (2) specific behavioral effects on operant response levels; and (3) electroencephalographic changes resulting from amphetamine administration.

The choice of drug was d-amphetamine sulfate, selected for its pronounced behavioral effect as reported by prior literature. Primary consideration to the relevant brain areas to be selected was based upon choice of areas known to be intimately involved in emotional behavior with some clarity of definition as to each area's involvement in processes thought to be excitatory or inhibitory in nature.

The brain loci chosen for stimulation were the basolateral amygdala, posterior hypothalamus, ventral hippocampus, and the centre median nucleus of the thalamus. The first two brain areas appear related to a system of excitatory activity, while the latter two areas are inhibitory in function.

The loci selected for electroencephalographic recording

were placements contralateral to the sites of the stimulation cannulae in addition to bilateral implantation in the motor, polysensory and visual cortices, as well as the lateral geniculate.

The task utilized was a visual discrimination go-no-go task in which the stimulus was cued by a flashing light and rewarded by a bar press. Asymptotic performance was achieved prior to initiation of experimental administration of the drug. Pertubations away from the established behavioral and electroencephalographic levels were considered indices of the effect of the experimental variable on brain processes.

CHAPTER II

REVIEW OF THE LITERATURE

This literature review is subdivided into three sections. The first is concerned with the structural elements of the anatomical regions considered in this experiment and their interconnections. The second section deals with operant and neuro-behavioral effects resulting from stimulation without regard to the anatomy of the area stimulated. The latter portion of this section focuses on the neuroanatomical regions specified in section one. The final segment is briefly concerned with pharmacological agents which may effect the electroencephalogram with the major emphasis of the review concentrated on the drug, amphetamine, its mode of action in the central nervous system, its structural properties, and its characteristic effects on behavioral patterns and the electroencephalogram.

Neuroanatomical Considerations

Amygdala

The amygdaloid complex is situated at the end of the inferior horn of the lateral ventricle. Surrounded by the periamygdaloid cortex of the hippocampal gyrus and bordered by the main body of the hippocampus at the posterior border, the

amygdala is positioned with the ventral part of the claustrum and putamem and continuous with the tail of the caudate nucleus. Two major nuclear groups constitute the major components of the amygdaloid complex. These are the corticomedial and basolateral nuclei.

The basolateral complex contains two subdivisions within itself-the lateral amygdaloid nucleus and the basal amygdaloid nucleus. The lateral amygdala is continuous with segments of the claustrum and the overlying cortical areas which accompany it. The basal amygdaloid nucleus is medial and dorsomedial to the lateral amygdala and is continuous with the overlying cortical matter. The accessory basal nucleus separates the basal and cortical nuclei and also is anatomically continuous with the overlying cortex. The basolateral nuclei has extensive projections to the corticomedial nuclei. The hippocampal and dentate gyri receive extensive afferents from it, establishing a major route between the amygdala and the posterior hypothalamus and midbrain tegmentum by way of the fornix, arising in part from the hippocampal gyrus(Grossman, 1967).

Hypothalamus

The hypothalamus, a part of the diencephalon, is bordered anteriorly by the lamina terminalis, above by the anterior commissure, and below by the optic chiasma. The hypothalamic sulcus lies dorsally, marking the junction with the thalamus. The ventral boundary is the tuber cinereum,

tapering into the infundibulum. The major structures laterally are the internal capsule, the subthalamic nucleus, and the basis pedunculi.

The posterior hypothalamus is located between the third ventricle and the subthalamic areas. In its caudal orientation, it is laterally bound by the mammillothalamic tracts. This nucleus is formed of small cell types indigenous to the anterior hypothalamic nucleus and the larger cells common to the lateral nucleus. These neurons also originate in the descending efferent pathways passing through the central gray matter as the dorsal longitudinal fasciculus and through the brain stem reticular formation (Netter, 1962).

Hippocampus

The hippocampal formation is a larger, complex archipallial structure occupying a major portion of the central section of each hemisphere. The lamina terminalis is the adjacent structure at its anterior extension. In lower animals the hippocampus extends posteriorly and dorsally to become continuous with the splenium of the corpus callosum. In the human brain the hippocampus extends from the septum over the corpus callosum and ventrally, adjoining the rostral component of the temporal lobe. The hippocampal formation is divided from the neighboring allocortex by the hippocampal fissure. Temporally, the fissure separates the hippocampal formation from the hippocampal gyrus; dorsally, it is continuous with the supracallosal sulcus which separates the

supracallosal gyrus (dorsal hippocampus) from the cingulate gyrus (Grossman, 1967).

Afferent imputs projecting to the hippocampus are the cingulum and olfactory fibers, as well as, an additional afferent initiated in the septal region and entering the hippocampus via the supracallosal stria into the dentate gyrus (Russell,1961). Cortical association afferents are the fibers from the posteromedial and inferior temporal region and the angular gyri (MacLean, 1954).

The fornix is the sole efferent pathway from the hippocampus. Allen (1944) and Fox (1943) observed that the precommissural fornix has a dorsal projection of fibers into the lateral septum. Additional fibers terminate in the preoptic nuclei region, including the diagonal band and nucleus accumbens (Fox, 1943; Simpson, 1952).

Electrophysiological research has provided delineation of the dorsal and ventral hippocampus. Elul (1964) showed that the ventral area innervates the amygdala, septal region, associational and specific thalamic nuclei, lateral and preoptic hypothalamic areas, and parietal and temporal cortices. It is influenced by the septum, amygdala, intralaminar and associational thalamic nuclei, as well as the caudate and putamen.

Thalamus

The thalamus is a large, diversified body of nuclei, too broad in scope to be of relevance to this literature review.

Consequently, the limit of this section will be confined to the dorsal thalamus, that area which occupies the center of the brain.

The thalamus can be divided into the following nuclear masses (Truex and Carpenter, 1964): the anterior nuclei, the midline nuclei, the lateral nuclei, the ventral nuclei, and the medial nuclei.

The anterior nuclei are divided into the anteromedial, anterodorsal, and anteroventral nuclei. The anteroventral nucleus is the most prominent in the brain. Afferents to all three nuclei are received from the mammillary bodies via the mammillothalamic tract and radiate fibers to the cerebral cortex. The anteromedial mucleus has fiber connections with the anterior portions of the cingulate gyrus and the inferior surface of the frontal pole. The anteroventral projects to areas 23 and 24 of the cingulate gyrus. The anterodorsal nucleus projects to the retrosplenial (area 29) of the cingulate gyrus (Grossman, 1967).

The midline nuclei, phylogenetically the oldest nuclei complex in the brain, is well developed in macrosomatic organisms, but tends to weaken or deteriorate in higher mammals. Several nuclear groups are well defined here. The area surrounding the third ventricle is subdivided into the anterior and posterior paraventricular nuclei. Afferent fibers of the cells scattered around the stria medullaris comprise the parataenial nucleus. Dorsal to this region is the intero-anterodorsal nucleus and directly in the center of the thalamus are

the rhomboid and central medial nuclei which constitute that area called the massa intermedia. The nucleus reuniens is the most ventral of the midline structures, extending posterior from the caudal border of the anterior tubercle to the center of the massa intermedia. Afferent connections of the midline nuclei are received from the spinothalamic tract, the trigemino thalamic tracts, and the medial lemniscus. Efferent outputs can be identified to many areas of the cortex, the hypothalamus, basal ganglia, and amygdala (Netter, 1962; Grossman, 1967).

The lateral nuclei are the pulvinar, posterolateral, and dorsolateral nuclei. The afferents extending to the pulvinar nucleus originate in the amygdala, preoccipital and occipital areas. Efferents project to the occipitotemporal and parietotemporal cortex. The posterolateral nucleus operates in reciprocity with surrounding nuclei in the thalamus, sending and receiving from many structures. Additional afferents have been located in corticothalamic radiations to the posterolateral nucleus from parietal and frontal cortices (Grossman, 1967).

The ventral nuclei can be divided into several large nuclear bodies. The posteroventral receive afferent fibers from the gracilis and cuneate segments of the medial lemniscus. Ventral and lateral spinothalamic fibers also terminate there, as do fibers of the quintothalamic tract. The posteroventral nucleus has fibers radiating to the paracentral and

postcentral areas of the cerebral cortex. Additional fibers are received from the motor cortex, with reported projections of the lateroventral nucleus to the motor cortex (areas 4 and 6). The rostral component of the ventral thalamus is the anteroventral nucleus. Afferents from the globus pallidus and frontal cortex have been reported; however, efferent fibers are poorly defined and not well identified.

The medial nuclei are the nuclear groups in the thalamus of principle concern for the current experiment. Their two major components are the dorsomedial nucleus and the centrum medianum. The dorsomedial nucleus extends in a caudal direction to the nuclei centrum medianum and parafasciculus and dorsally to the anterior nuclei. This nucleus has reciprocal afferent-efferent systems with the hypothalamus, pretectal region, the prefrontal cortex, and other thalamic nuclei (Truex and Carpenter, 1964).

The centrum medianum divides the red nuclei from the dorsomedial and lateral nuclei. It is the largest nucleus in the intralaminar group. The nuclei receives retriculothalamic fibers from the brainstem tegmentum and is the origin of a major portion of the intrathalamic fasciculus (Jasper and Ajmone-Marsan, 1961). The intrathalamic fasciculus joins the medial nuclei to the ventromedial portion of the ventral anterior nucleus (Nauta and Whitlock, 1954). Efferents radiate to all the intralaminar bodies from the fasciculus, as well as the lateral association areas. Efferents also project

to the reticular nucleus, basal ganglia and hypothalamus. Afferents are poorly defined but are apparently received from secondary motor and sensory pathways (Russell, 1961).

Neurobehavioral Effects of Drugs

A broad spectrum of drugs have been utilized to affect emotional behavior and produce facilitation or inhibition of learning on behavioral tasks. This section first examines several categories of these agents in general, then explores research related to the neuroanatomical region specified in section one.

Analeptics

Facilitation of learning has been obtained with low doses of a number of analeptic drugs, among them strychnine, picrotoxin, and pentylenetetrazol (Metrazol).

Strychnine sulfate was first used by Lashley (1917) in a study in which he found maze learning in rats was enchanced by daily pre-test injections of the substance. This effect has since been replicated by McGaugh and Petrinovich (1959) and Petrinovich (1967). The facilitation effect of strychnine holds valid across species, including cats and monkeys in a wide variety of learning tasks. Its action has been shown in discrimination learning (McGaugh and Thompson, 1962; Petrinovich, 1963), escape and avoidance learning (Keleman and Bovet, 1961), and classical conditioning (Benevento and

Kandel, 1967).

Krivanek and McGaugh (1968) administered pentylenetetrazol to mice in subconvulsive doses each day immediately following testing on a discrimination task. The results indicated the experimental groups improved; however, the degree of facilitation of learning was a function of the dose strength of the pentylenetetrazol. In a range of levels from 2.5mg/kg of body weight to 20.0 mg/kg, the most effective dose was 15.0 mg/kg. Krivanek (1968) found that in avoidance learning, the dose response level varies with a number of parameters; facilitation is possible with many conditions, but the magnitude of improvement for a particular dose can vary with sex, shock level, and level of food deprivation. Krivanek and McGaugh (in press) have also shown that on a maze-learning task, the optimal dose level for facilitation varies for different strains of mice.

Picrotoxin produces facilitation in learning similar to that which occurs with strychnine and pentylenetetrazol. Post-training injections in mice and rats have produced positive effects (Breen and McGaugh, 1961; Bovet et al., 1966; Zerbolio, 1967). Picrotoxin has also been shown to enhance a conditioned photic response (plotnikoff and Evans, 1967).

Parasympathetic Depressants

Scopolamine, a known antagonist to the parasympathetic effects of acetylcholine, has been recently shown by Leaf and Muller (1966) to facilitate learning of a free operant avoidance task if it is given in small doses during a task

that has early massed trials. Hearst (1964) also utilized scopolamine on a reinforcement schedule in which monkeys could postpone electric shock. These periods occurred only in the presence of a light of a specified intensity. The monkeys exhibited the generalization phenomena when exposed to lights of other intensities-they responded at lower rates. Hearst then showed that in the presence of stimuli similar to the type used in original training, response levels were less affected than it was when the animals received experimental stimuli dissimilar to the training lights. Scopolamine increased responding more in the presence of the dissimilar stimuli.

Atropine, another cholinergic blocking agent, can be either an enhancer or depressor. When applied to the midline nuclei of the thalamus, it produced facilitory effects on behavior in both appetitive and aversive testing situations. However, when administered to the reticular nuclei of the thalamus, atropine produced a marked performance deficit during acquisition and at asymptotic level on both an appetitive and avoidance learning task (Grossman and Peters, 1966).

The crux of the literature reported within this section to this point is that a wide spectrum of variables exist which appear to interact when drugs are administered in a behavioral task. Standardization of the literature to resolve these categories is far too lengthy and involved to be accomplished within the limits of this review. The

explicit effects of these more recent drugs applied directly to subcortical structures is relatively untested; therefore the balance of this second section is devoted to the characteristic effects resulting from chemical and electrical stimulation within the neuroanatomical regions specified in section one of the literature review.

Amygdala

Grossman (1964) reported that adrenergic stimulation of the ventral amygdala in water-deprived cats facilitated lever pressing for food reward, but depressed operant behavior rewarded by water. Cholinergic blocking agents (i.e. atropine sulfate) produced response and behavioral patterns similar to adrenergic stimulation. Cholinergic stimulation in the basolateral amygdala precipitated long term epileptiform seizures and visible motor seizures shortly after injection (Grossman, 1963).

Hypothalamus

Chemical activation or inhibition was utilized by Grossman (1960, 1962) to selectively facilitate or inhibit the performance of food-or-water-motivated responses. However, Grossman felt that these results reflected motivational changes rather than alteration of the level of association for the subject. Grastyán et al. (1956) reported that electrical stimulation of the dorsolateral hypothalamus of the cat facilitated a simple instrumental response for food reinforcement; however, stimulation of the posteromedial hypothalamus and

posterior portion of the mesencephalic reticular system inhibited the food-motivated response. Roberts (1958) tested the posterior hypothalamus in comparison with a variety of nuclei, including the medial lemniscus and ventral part of the thalamic nuclei. Stimulation of the posterior hypothalamus elicited escape behavior, but did not facilitate avoidance learning. Stimulation of the other structures mentioned facilitated both escape behavior and avoidance learning.

Hippocampus

Clear separation of loci of behavioral change to stimulation in the hippocampus is poorly defined. Due to often inadequate control over motivational parameters, a clear decision as to the extent that the associational processes are involved is difficult. However, electrical stimulation of various hippocampal structures has been shown to contain positive reward aspects (Delgado et al., 1954) as well as improve performance on food-reinforced tasks (Correll, 1957). MacLean et al. (1955) discovered that cats and monkeys fail to retain a previously learned avoidance behavior when seizures are precipitated in the hippocampus by carbachol injection. The loss did not seem to be motor function as the subjects were capable of escape at the onset to the UCS. Flynn et al, (1961) blocked a priorly-learned leg flexion by electrically inducing hippocampal seizures.

Thalamus

The literature of thalamic stimulation, either chemical

or electric, is sparce. Much of the research reported has been directed to the large anterior nuclei. Maire (1956) and Smith (1967) have observed that electrical stimulation to the anterior nucleus of the thalamus in the rat produced seizure activity and ravenous eating. However, food reinforcement was insufficient reward to facilitate bar pressing behavior. In a well-controlled experiment by Roberts (1958) the effects of central "flight" stimulation was compared with the effects of central "alarm" and peripheral pain stimulation. Flight behavior was localized to a small area in the posterior hypothalamus. Alarm reactions were produced by stimulation in the ventro posterior nucleus of the thalamus. Peripheral pain and central alarm stimulation facilitated both escape and avoidance learning. Endroczi et al. (1959) trained dogs to push a door (CR) when a bell was presented (CS) and withhold the CR in the presence of any other auditory stimulus. Electrical stimulation of the centre median nucleus seemed to facilitate the CR. The stimulation period produced pleasure reactions in the animals as well. However, Grossman et al. (1965) found that cholinergic stimulation in the thalamic midline nuclei of rats impaired simple shuttle box avoidance performance. Cholinergic stimulation of the reticular nuclei of the thalamus depressed asymptotic performance as well as acquisition. Cholinergic stimulation with carbachol directly to forebrain structures (Hull et al., 1967) produced a consistent depression of response in a bar-pressing situation on a FR schedule.

The aforementioned research is a cross-section of behavioral

findings related to stimulation designs. The effect of psychopharmacological agents on central nervous system activity is the topic the following section.

Drug Stimulation and Subsequent Electroencephalographic Change

The following section is a brief analysis of various drugs in relation to their effects on the elctroencephalogram, followed by an extensive review of the pharmacological agent, amphetamine, and its effects on release of neurohumoral agents.

Selected Drugs

The effects of acetylcholine,arecoline,and pilocarpine, cholinergic agonists, show a characteristic pattern on the electroencephalogram when administered intravenously to a subject-the background slow wave electroencephalographic pattern shifts to an activation response in the neocortex and limbic system (Drew and Domino, 1968; Yamoto and Domino, 1967). Administration of physostigmine and nicotine also produce similar activation patterns. Both of the above experiments blocked the agonists with methyl atropine and found a reversal in the hypotensive condition produced by the cholinergic agents, but not a blockade of the activation of the electroencephalogram. Atropine sulfate however, blocked both hypotension and electrical activation patterns. Injections of carbachol, a cholinergic agonist, directly into forebrain

structures (Hull et al., 1967) during a bar-pressing task produced activation patterns similar to those reported above. Typically, the electroencephalogram was characterized by low voltage, fast frequency activity, broken by transient 6-8 c/sec high amplitude waves recorded from the visual cortex in the time interval following milk reinforcement to the animal.

Injections to the lateral hypothalamus of the adrenergic substances, epinephrine or norepinephrine, produced output similar to the cholinergic drugs. Fast frequency, low amplitude waves were seen (Grossman, 1964). The selectivity to behavioral response of norephinephrine injected directly into the lateral hypothalamus is interesting. Such administrations elicited feeding, while interperitoneal injections elicited no feeding response. However, carbachol administered to the same site produced not feeding, but drinking behavior (Miller, 1965).

Amphetamine

Various theories on the mechanism of the central nervous system stimulation properties of amphetamine have been proposed over the last thirty years. Mann and Quastel (1940) suggested that amphetamine acted by inhibiting monoamine oxidase(MAO). By 1950, this theory was weakening as new and potent MAO inhibitors were developed. Amphetamine actions were distinctly different from those clearly defined MAO inhibitors. When the central action effect of amphetamine

persisted after MAO had diminished a theory arose that it acted directly on serotonin (5-HT) mechanisms (Vane, 1960) and on catecholamine receptors (Van Rossum et al., 1962). A successor to the aforementioned theory was a hypothesis that amphetamine mimicked norepinephrine (NE) and combined directly with the norepinephrine receptors in the brain (Brodie and Shore, 1957; Smith, 1963).

The preliminary reports suggesting an indirect action of amphetamine dependent upon the presence of brain monoamines were suggested by Quintow and Halliwell (1963) and Stein (1964).

In 1965, the theory was independently validated by Glowinski and Axelrod. Stein (1967) and Stein and Wise (1969) have elaborated the hypothesis with the suggestion that the behavior-facilitation effect of amphetamine is dependent upon the availability of NE in functional pools (those participating in synaptic transmission) rather than reserve pools (seemingly unrelated to transmission). The most current findings suggest that norepinephrine released by amphetamine acts primarily as an inhibitor at the cellular level, depressing activity of behaviorally-suppressive cell groups in the forebrain bundle (Stein and Wise, 1969).

Structural properties. Amphetamine is a rather unique drug possessing a simplified structure and a multiplicity of biological effects. All of its structural elements are essential to its full biochemical spectrum. Figure 1 with its

accompanying identifications will be utilized to describe these characteristics.

The basic skeleton of amphetamine is that of a sympathomimetic amine. For that reason, the β-phenethylamine grouping (3) is critical the pharmacological property of release of norepinephrine (NE) from functional neuronal storage sites and subsequent blockade of re-uptake of membranal NE (Daly, Creveling and Witkop, 1966; Iverson, 1967). Any type of substitution in the phenyl ring (1) will eliminate or alter the characteristics of its central effects. Group (2), the α-methyl structure protects amphetamine from MAO destruction. Its inhibition characteristics on MAO are considered to be mild to moderate (Glowinski, Iverson and Axelrod, 1966). It also possesses a strong affinity for the membrane uptake mechanism and subsequent blockage of NE at the membrane (Iverson, 1967).

Methylization of either the primary amino group (4) or the side chain (3) decreases the characteristic action of amphetamine at a rate equivalent to the degree of methylization. Shift of the α-methyl group to the β-position will virtually abolish all central and anorexic (food intake blockade) characteristics of amphetamine (Daly et al., 1966).

Behavioral Effects. Evidence is strong that amphetamine can improve operant levels on a behavioral task if certain precautions are met. Stein (1964) stimulated the medial forebrain bundle electrically and with amphetamine and produced retardation of extinction of food reinforced behavior, as

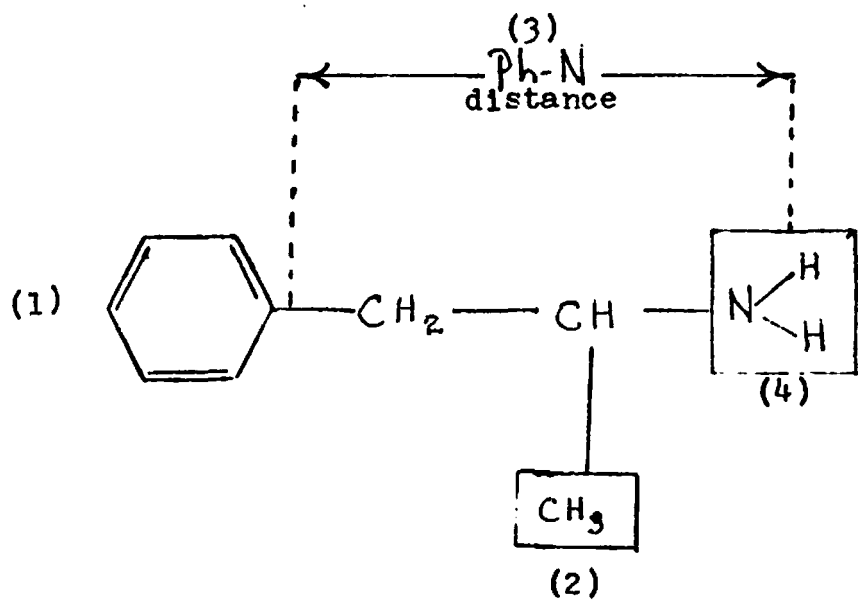

Fig. 1. Structural moieties critical to amino activity.
(1) an unsubstituted phenyl ring
(2) the α-methyl group
(3) a 2-carbon side chain between the phenyl and amino radicals
(4) the primary amino group (Biel, 1970)

well as facilitation of performance in a shuttle-box avoidance task. Brady (1958) found that amphetamine will speed up low response rates in a schedule in which only low rates are reinforced (DRL) and Pickens and Harris (1968) successfully trained monkeys and rats to perform a task for intravenous injections of amphetamine, much as they would for electrical stimulation of the medial forebrain bundle.

Electroencephalographic characteristics of amphetamine have been universally reported as a shift to low-amplitude, high frequency brain activity (Schallek et al., 1967; Drew and Domino, 1968; Khavari, 1969). The following study by Cahn and Herold (1970) is a brief index of the changes in brain activity attributable to amphetamine compounds in the rabbit.

Methamphetamine administered intravenously produced desynchronized, fast activity which was more evident in the sensorimotor cortex than in posterior leads. Various blocking agents were administered following injections of methamphetamine to reduce the amphetamine effect. Phentolamine synchronized the brain activity; however, propranolol did not exert a pronounced effect.

Fairchild et al. (1967) examined the effects of amphetamine and four-ring substituted amphetamine derivatives on spontaneous electrical activity in the cat. Animals injected interperitoneally with the amphetamine analogs displayed initial restlessness, accompanied by occasional urination, defecation and vomiting. After the initial

stages abated, the animals generally remained in a prone position-head erect, forelimbs rigidly extended. The animals appeared highly alert, but seldom moved. All characteristic symptoms of sympathomimetic stimulation were observable-mydriasis, hypernea, pilo erection, etc. Coincident with this behavior was a brain pattern of high-amplitude, low frequency waves, characterized by hypersynchronous rhythms in the frequency range of 3 to 10 c/s.

Amphetamine, in contrast to its analogs, produced only a relatively flat, desynchronized electrical activity characterized by an increase in general activity. The qualitative analysis of the electroencephalogram indicated a general decline in the lower frequencies and a moderate gain in the higher ranges under amphetamine.

CHAPTER III

METHODS AND PROCEDURES

Subjects

The subjects were eight mature male cats, each animal weighing between eight and eleven pounds. The animals were maintained independently in stainless steel cages in the vivarium of the Psychology Department at the University of Houston. Feline distemper was administered each animal upon its acquisition and successive dosages of 300,000 units of Crysticillan were given daily beginning three days prior to surgery and terminating three days after surgery. All animals were maintained on Purina dry cat food and water with milk supplement acquired in testing sessions.

Electrodes

Bipolar electrodes for both cortical and sub-cortical placement were constructed in the research laboratory. Each electrode pair was constructed of two pre-insulated 30-gauge nichrome wires stretched and alligned with one tip extended one millimeter farther than the other. These wires were then dipped in Epoxylite electrode insulator and subsequently baked in an oven for five minutes at 375 F. This procedure was repeated three times to insure adequate insulation within each bipolar assembly. Each electrode was then scraped at each tip to allow 0.5 millimeter exposure. The two leads above the shaft were incased in teflon tubing

and crimped with gold connector pins, designed for insertion into an AMP connector, the details of which are found elsewhere (Grandstaff, 1965).

Cannulae

The cannulae used were constructed in the laboratory and consisted of an external, indwelling cannula and an internal cannula used to administer the experimental drug and saline.

The external cannula was composed of a 20 gauge stainless steel tube and the internal brass screw-in connector from a Harris #211 banana plug. The shaft was cut to its selected length, then the brass connector was anchored to its exterior with Eastman 910 adhesive cement. To insure adequate fit between the shaft and connector, a small length of polyethylene tubing was inserted as a spacer.

The injection cannula was composed of a 24 gauge stainless steel syringe with a small, brass jeweler's spacer glued to its exterior, and a small section of 20 gauge stainless steel tubing. The jeweler's spacer served as a control for depth so that the injection cannula could penetrate no farther than 2 mm beyond the tip of the external cannula. The 20 gauge tubing was glued to the 24 gauge tubing above the spacer to assure adequate snugness between the injection cannula and the polyethylene tubing leading to the microsyringe.

Dummy injection cannulae constructed of 24 gauge stainless steel wire were cut to the same length as the injectors

and maintained in the indwelling cannulae at all times other than injection periods.

Surgery

All surgical instruments and stereotaxic components were cleansed and placed in a Zephiran chloride solution (.13%), 24 hours before surgery.

The anesthetic used was a 65% solution of Vetinary sodium pentobarbitol, administered in a dose corresponding to one cc. per five pounds of body weight. The principle injection site was the liver with the alternative of infusion from the peritoneal cavity.

The scalp of each animal was shaved and cleansed with both Phisohex and alcohol. A local anesthetic, 2.5% solution of procaine hydrochloride, was injected subcutaneously in the scalp region. The animal was then mounted in a Johnson cat stereotaxic apparatus.

A 50 mm medial-sagital incision was made on each animal exposing the cranial bone, which was then laterally stripped of periostium. The overlying superior temporalis musculature was removed to allow 15 mm of unobstructed cranial bone laterally to each side of the medial sagittal suture. The cranium was then cleansed with saline.

The stereotaxic placements were marked on the cranium bilaterally and the skull trephined with a dental burr. Four cannulae and four pairs of bipolar electrodes were implanted in the right hemisphere of the brain. Eight pairs of bipolar electrodes were implanted in the left hemisphere. The

coordinates for placement of cannulae and electrodes were obtained by a synthesis of atlas coordinates determined by Reinoso-Suarez (1961) and Jasper and Ajmone-Marsan (1954). The coordinates for cannula in the right hemisphere were as follows: ventral hippocampus, 9.5 mm lateral, 9.0 mm anterior, -5.5 mm horizontal; posterior hypothalamus, 1.5mm lateral, 10.0 mm anterior, -6.0 mm horizontal; basolateral amygdala, 9.0 mm lateral, 12.0 mm anterior, -5.5 mm horizontal; and centre median nucleus of the thalamus, 3.0 mm lateral, 7.0 mm anterior, 1.0 mm horizontal. Bipolar electrodes were implanted in contralateral positions to the cannulae in addition to the following placements: lateral geniculate, 9.5 mm lateral, 7.0mm anterior, 13.0 mm from dura; visual cortex, 5.0 mm lateral, 10.0 mm posterior; motor cortex, 7.0 mm lateral, 24.0 mm anterior; and anterior suprasylvian cortex, 10.0 mm lateral, 15.0 mm anterior. All placements were zeroed on the interaural canal with the exception of the lateral geniculate whose depth was calculated from the dura.

Nu-Weld dental acrylic was used as the bonding agent for cannulae and electrodes. All electrodes leads were secured in an AMP connector which was, in turn, secured by additional acrylic. The plug was further secured to stainless steel wire anchored by trephinations in the frontal sinus and cerebellar region.

The scalp was then cleansed and closed with nylon sutures anteriorly and posteriorly to the dental acrylic. As a pre-

ventive measure, each animal was given 450,000 units of Crysticillin post-operatively for three days.

Chemicals

The drug used was d-amphetamine sulfate suspended in distilled water with an additive of 1.5% benzyl alcohol per cc. of solute as a preservative. The drug was prepared and distributed by Spencer-Mead Company of Valley-Stream, New York with a strength of 20 mg per ml. of solute. Proper dose levels were obtained emperically by preliminary pilot research on two animals. Pre-pilot levels of two mg/kg of body weight for systemic effect and 40μg per animal for intracranial stimulation were established based upon reported levels by Khavari (1969), Norton (1967), and Fairchild, et al. (1967). The accepted dosages for this design following pilot work were one mg/kg of body weight per animal delivered in a volume of 1mg/5μl solute for all inter-peritoneal injections. Intracranial levels were set at 80μg of amphetamine sulfate delivered in a volume of 4μl solute. These levels produced adequate general behavioral effects with minimal interference with the experiment task.

Experimental Apparatus

Microsyringe. A Hamilton 100 microliter syringe #710 was used as the vehicle for injection of the amphetamine sulfate. A vernier calibrated micrometer was mounted in a metal stand in conjunction with a calibrated syringe barrel. Adjusted calibration allowed 1μl of solute to pass for each

1.3 revolutions of the micrometer. Three feet of teflon tubing were attached to the microsyringe and capped on the open end with the injection cannula.

Testing Chamber. The testing chamber was constructed of white Formica with the dimentsions 48"x30"x36". The entire chamber was electrostatically-shielded and sound-proofed. One wall was entirely composed of one-way glass to allow observation of animals at all times. Lighting was provided by a Foringer-controlled pair of D-C lamps installed within the chamber. An additional D-C light source was installed to provide a photic stimulus for testing. Directly beneath the photic stimulus lamp was a liquid feeder system which worked in conjunction with a Skinner bar. Immediately above the photic lamp opening was an audio speaker.

Liquid Feeder. The mode of delivery of liquid reinforcement was a siphon liquid feeder system controlled by a Skinner Electric Valve Company solenoid valve operating on 24 votts d.c. The valve had an internal diameter of .25 inch with a rating of 10 psi. Tubing above the valve extended into the receptacle containing the liquid reinforcer. This container was located above and adjacent to the experimental chamber. The tubing below the solenoid connected into the chamber and emptied its contents into a feeder cup adjacent to the Skinner bar.

Operation of the valve was directed by two options: (1) the Skinner bar when depressed closed a microswitch, and in doing so, delivered current to the valve which in

turn released .3 cc. of liquid to the feeder cup; or (2) a manual control switch was available to the experimenter which, when depressed, closed the microswitch allowing any desired amount of liquid to flow into the feeder cup.

Photic Stimulator. A Grass model PS2 photic stimulator, explicitly designed for psychophysical and neurophysiological research, was used. The amplifier for the photic driver was located with accompanying Foringer equipment in a room adjacent to the testing chamber. The apparatus, driven by 24 volts d.c., possessed a variable control for both intensity and frequency per unit time of the photic flashes. A controlled 4 cycles per second setting with an intensity level of eight was held constant throughout this experiment.

Control and Recording Equipment. All contigencies were automatically controlled by the use of Grayson-Stadler operant behavior equipment. A Rheem tape reader, capable of reading an eight channel tape was utilized to introduce all reinforcement contingencies. The contingencies were contained on perforated tape which was read by photoelectic cells in the reader. Trials and responses were automatically tabulated by a series of digital counters.

Electroencephalograph. A seven analog and two-event channel Grass, Model III-D, electroencephalograph was used as the basic recording unit. The seven channel of analog signals recorded seven selected pairs of electroencephalographic responses. The two digital channels recorded

subject responses and stimulus periods onset and duration. Operating in parallel with the Grass was a seven-analog and eight-digital channel magnetic tape recorder which permanently stored the signals on tape. The principle analog channels were motor cortex, polysensory cortex, basolateral amygdala, posterior hypothalamus, lateral geniculate, ventral hippocampus, and visual cortex. The digital channels stored were indices of subject bar presses and onset and duration of the stimulus period and pre-stimulus period.

Experimental Procedure

Preliminary Training. All animals were maintained on 23 hour water deprivation, ad lib water being available for one hour following each daily experimental block of trials. Diluted canned Pet evaporated milk served as liquid reinforcer throughout the experimental sessions. By a method of successive approximation, as well as experimenter-guided motor movement, all subjects were shaped to bar press for milk reinforcement. Initial training included a secondary cue, a 500 cps tone, which occurred coincident with each bar press until the subject made 20 successive bar presses. Upon attaining the shaping criterion, the auditory cue was eliminated, the illumination in the experimental chamber lowered, and a continuous reinforcement schedule(CRF) begun for the subject.

The CRF schedule was maintained for two days and then expanded on the third day to a fixed-ratio (FR) schedule with reinforcement on each third response. The FR-3 schedule was

maintained for two days and then phased to a FR-5 schedule which was maintained for six days.

Phase I was then initiated which consisted of 50 daily trials presented on a varied interval schedule with a mean inter-trial interval (ITI) of 30 seconds. Each trial consisted of a 10 second S_D period, cued by a 4 c/sec. flashing light, during which the animal was reinforced for each bar press. These daily sessions were continued until each animal met a criterion of twice as many S_D responses as ITI responses, at which point the subsequent daily sessions were examined for evidence of asymptotic performance on the task. The index of performance examined was the ratio of ITI responses divided by S_D responses. When this ratio stabilized for six successive days, the subject was begun on the experimental variable.

Experiment I. One day prior to introduction of the experimental variable, a 50 trial session was run in which EEG measures used as baselines were obtained from each animal.

The intent of the experiment was to measure EEG and behavioral changes resulting from injections of d-amphetamine sulfate into the basolateral amygdala, posterior hypothalamus, ventral hippocampus, and centre median nucleus of the thalamus as well as a systemic injection administered intraperitoneally. Saline administration was used as a control condition. Each animal served as his own control and received all six conditions. The sequence of administration

was balanced in a latin-square design, the orderings of which can be seen in Appendix A. The saline condition was randomly distributed among each of the experimental conditions as well.

A 72-hour separation was used between injections to allow for dissipation of effects from prior stimulations. On injection days one of two procedures was used dependent upon the nature of the stimulation: (1) an intracranial injections of 80 μg(4 μl) of amphetamine sulfate was administered, or (2) 1 mg/kg of body weight of the animal was injected into the intraperitoneal cavity of each animal.

On those injection days necessitating intracranial stimulation, the stainless steel dummy cannulla was withdrawn, the injection cannula inserted, 4 μl of solution injected, and the dummy cannula replaced. The animal was then immediately placed within the experimental chamber to begin a block of 50 trials on Phase I, during which general behavioral observations were taken, as well as operant levels of responses, and EEG recordings.

On systemic injection days, the drug was administered and allowed one hour to take effect. The animal was then placed in the experimental chamber and the trials begun. Response measures were the same as those listed for intracranial injection.

Following each injection day, blocks of 50 trials were run at 24 hours and 48 hours with behavioral and operant

measures obtained.

Experiment II. Following completion of Experiment I, partial replication of the original design was executed by repetition of intracranial stimulation of the basolateral amygdala for each of the last three animals in the design. Again behavioral observations, operant levels, and EEG recordings were obtained. Twenty-four and 48 hour measures were taken as described in Experiment I.

CHAPTER IV

RESULTS

Presentation of the results of Experiments 1 and 2 is divided into four sections. The initial section is a histological substantiation of electrode and cannula placements. The second section is an analysis of the general behavioral characteristics observed during amphetamine administration and testing of all experimental conditions. Section three deals with statistical analysis of the operant behavioral data, as well as, notations regarding general operant trends or patterns. The final segment is a comprehensive examination of the effects of amphetamine on the electrical activity of the brain. This analysis will quantitatively analyze the electroencephalogram for alterations or trends in specified frequency bands.

The statistical tests utilized for evaluation of the operant behavioral responses were an extension of a Freidman rank test, in which several samples may be compared against a control or standard group, and the Walsh test for related samples.

Histological Evaluation

Cats 2,7,8,9,10,and 11 were sacrificed and histologies

performed on their brains. Each animal was decapitated and its head immersed in a receptacle of 20% formalin for 72 hours. The cranial bone was then stripped away to allow the formalin more facility of access to the brain tissue. After 48 additional hours, each brain was blocked and removed from the skull. The block was then placed on the stage of a microtome and frozen with carbon dioxide. Sections were taken and evaluated for cannula and electrode placements with the following results:

(1) Location of the cannula tips in the amygdala were accurate within $\pm$1.5 mm anterior-posterior and $\pm$1.0 mm lateral to the coordinates listed in the methodology chapter. Horizontal plane coordinates were accurate to $\pm$ 1.0 mm. All cannulae tips were within the amygdala nuclei. Amygdala electrodes were displaced to roughly the same degree of error.

(2) Posterior hypothalamic cannulae were accurate within $\pm$1.0 mm anterior-posterior, $\pm$.5 mm lateral, and $\pm$.5 mm horizontal to the intended coordinates.

(3) Centre median cannulae and electrodes were displaced by a much as $\pm$1.5 mm in each plane with a tendency to over extend the lateral placements.

(4) Hippocampal cannulae were displaced slightly toward the mid-line and generally located at the tip of the optic tract. Consequently errors in placement were as great as 1.5 mm proximal to the mid-line. Horizontal placements were generally 1.5 mm dorsal of the intended location. Electrodes

were positioned with a similar degree of error.

General Behavioral Observations

Several behavioral characteristics were common to both systemic and intracranial stimulation. Following administration of the drug and initiation of the experimental trials, all animals positioned themselves in a selected position in the experimental chamber confronting the photic stimulus and seldom moved through the 37-minute testing period. Their activity during this time was characterized by a rigid upright body position. Major body motion was confined to lateral movement of the head as if to attend to a sudden stimulus.

Systemic Stimulation

Pilot tests on interperitoneal injections indicated the use of 2 mg/kg of amphetamine sulfate resulted in attenuation of the conditioned response on the operant task. All animals appeared alert and responsive, but failed to bar-press during testing and at 24 hours post-stimulation. Reduction of the dose level to 1 mg/kg reinstated bar-pressing behavior at 24 hours. Both dose levels produced the full amphetamine reaction mentioned in Section II. All animals displayed evidence of mydriasis, tachycardia, pilomotor contraction and hypertention.

Experimental trials within Experiment 1 were executed with the dose levels established at 1 mg/kg for all intra-

peritoneal injections. The resultant effects were identical to those reported in the pilot study. The visible behavioral effects of these stimulations persisted for approximately 24 hours from the time of administration, although operant effects were observed up to 48 hours.

Intracranial Stimulation

The behavioral effects of intracranial stimulation with 80 micrograms (.4g) of amphetamine sulfate were less severe than those characteristics observed in the systemic injections. Mydriasis was less severe, tachycardia present but milder, and hypertension was visibly reduced for stimulation in all four brain areas.

Operant Behavioral Results

The operant data from this experiment were quantified by reduction of each day's performance to a ratio based upon the following formula:

$$\text{ratio} = \frac{ITI_R}{S_{D_R}}$$

in which ITI_R was the number of non-reinforced responses made in the inter-trial intervals during testing, and S_{D_R} was the total number of reinforced responses made during the stimulus period.

Individual differences among performances of the animals provided a wide dispersion of ratios. Analysis of these ori-

ginal values would have been, at best, rather meaningless. Therefore a transformation was done on all experimental ratio scores in the following manner:

$$X_{trans.} = \frac{\overset{(1)}{\text{Baseline ratio}} - \overset{(2)}{\text{Experimental ratio}}}{\underset{(3)}{\text{Standard Deviation}}}$$

where the baseline ratio (1) was the mean ratio for performance on the last six days prior to onset of experimental testing; the experimental ratio (2) was performance on a specified day of interest, and the standard deviation (3) was calculated on the ratios of the six baseline values. The subsequent result was a standardized scale with a mean pre-test level of zero and a variance of one for all subjects. The mean and standard deviation values for all cats are found in Table 1.

Saline Administration

Saline administration, randomized in its placements sites as indicated in Appendix A, produced operant results similar to non-stimulation levels. Statistical analysis of saline days against baseline days proved to be non-significant.

Systemic Administration

Table 2 summarizes the results obtained with systemic injections of amphetamine sulfate. The data are presented in standardized ratios and appear graphically in Figure 2. Measurement units were departures from the mean pre-stimu-

TABLE 1

MEANS AND STANDARD DEVIATIONS OF BASELINE RATIOS

CAT	MEAN	STANDARD DEVIATION
2	.165	.037
7	.582	.079
8	.93	.128
9	3.120	.274
10	.445	.037
11	.582	.079

TABLE 2

SYSTEMIC STIMULATION WITH SUBSEQUENT RATIOS AT 24 AND 48 HOURS POST-STIMULATION

CAT	STIM. DAY	24 HRS.	48 HRS.
2	No response	.21	.09
7	No response	.31	.32
8	No response	No response	.61
9	4.70	1.83	1.97
10	.32	.44	.49
11	No response	.34	.42

FIGURE 2

PERFORMANCE FOLLOWING CENTRAL STIMULATION

lation levels, taken for the saline control day, stimulation day, and 24 and 48 hours following stimulation. As can be seen, subject performance on the saline control day was random with little variance. However, administration of the drug depressed performance on the stimulation day for four of the six animals. A consistent pattern evolved for performance at 48 hours. Increases in standarized ratios at 24 hours was non-significant; however, performance at 48 hours was significant at $P < .05$.

Intra-cranial Administration

Amygdala. Results of amphetamine sulfate stimulation of the amygdala is shown in original form in Table 3. Figure 3 represents the data transformations. Injections in the amygdala were characterized by large performance deviations from baseline levels as this figure indicated. Performance levels were significantly improved at 24 hours ($P < .05$) and 48 hours ($P < .01$).

Posterior Hypothalamus. Performances resulting from hypothalamic stimulation are represented in original form in transformations in Table 4 and Figure 4. Again, the stimulation day performances were extremely variable; however performance improved at 24 hours ($P = .06$) and 48 hours ($P = .06$).

Hippocampus. Performances resulting from hippocampal stimulation with amphetamine sulfate were non-significant at all levels.

Centre Median of the Thalamus. Performance levels for

TABLE 3

AMYGDALA STIMULATION WITH SUBSEQUENT RATIOS AT 24 AND 48 HOURS POST-STIMULATION WITH REPLICATIONS ON CATS 9, 10, AND 11

CAT	AMYG. STIM.	24 HRS.	48 HRS.
2	.22	.06	.07
8	1.00	.92	.76
9	4.55	2.12	1.79
10	.10	.32	.24
11	1.72	.46	.42

REPLICATION

CAT	2nd STIM.	24 HRS.	48 HRS.
9	2.34	1.52	1.85
10	.26	.40	.42
11	1.70	.59	.49

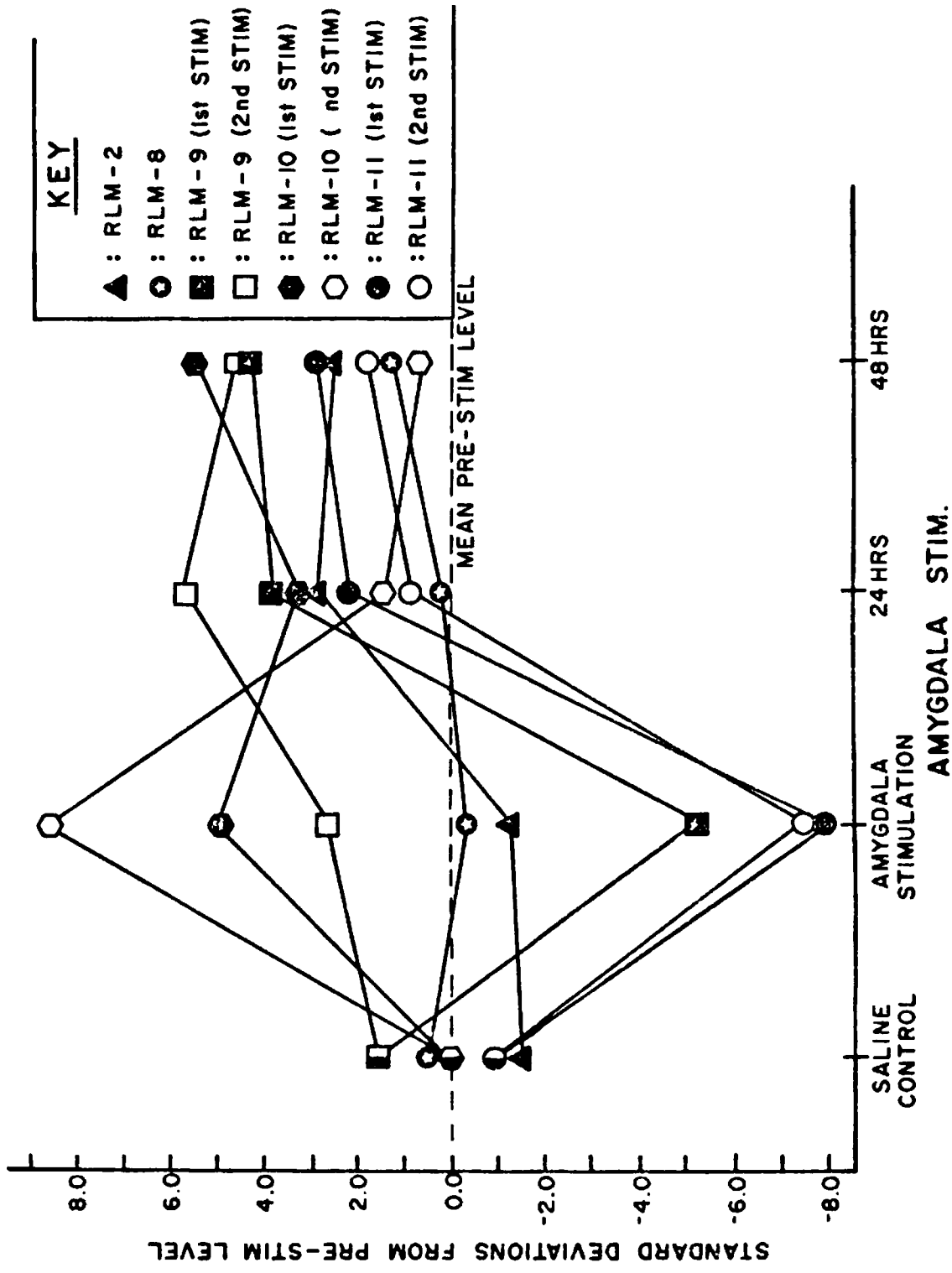

FIGURE 3

PERFORMANCE FOLLOWING AMYGDALA STIMULATION

TABLE 4

POSTERIOR HYPOTHALAMIC STIMULATION WITH SUBSEQUENT RATIOS AT 24 AND 48 HOURS POST- STIMULATION

CAT	HYPOTHALAMIC STIM.	24 HRS.	48 HRS.
2	.13	.04	.08
8	1.42	.97	.46
9	4.69	1.82	2.04
10	No response	.23	.18
11	.47	.67	.78

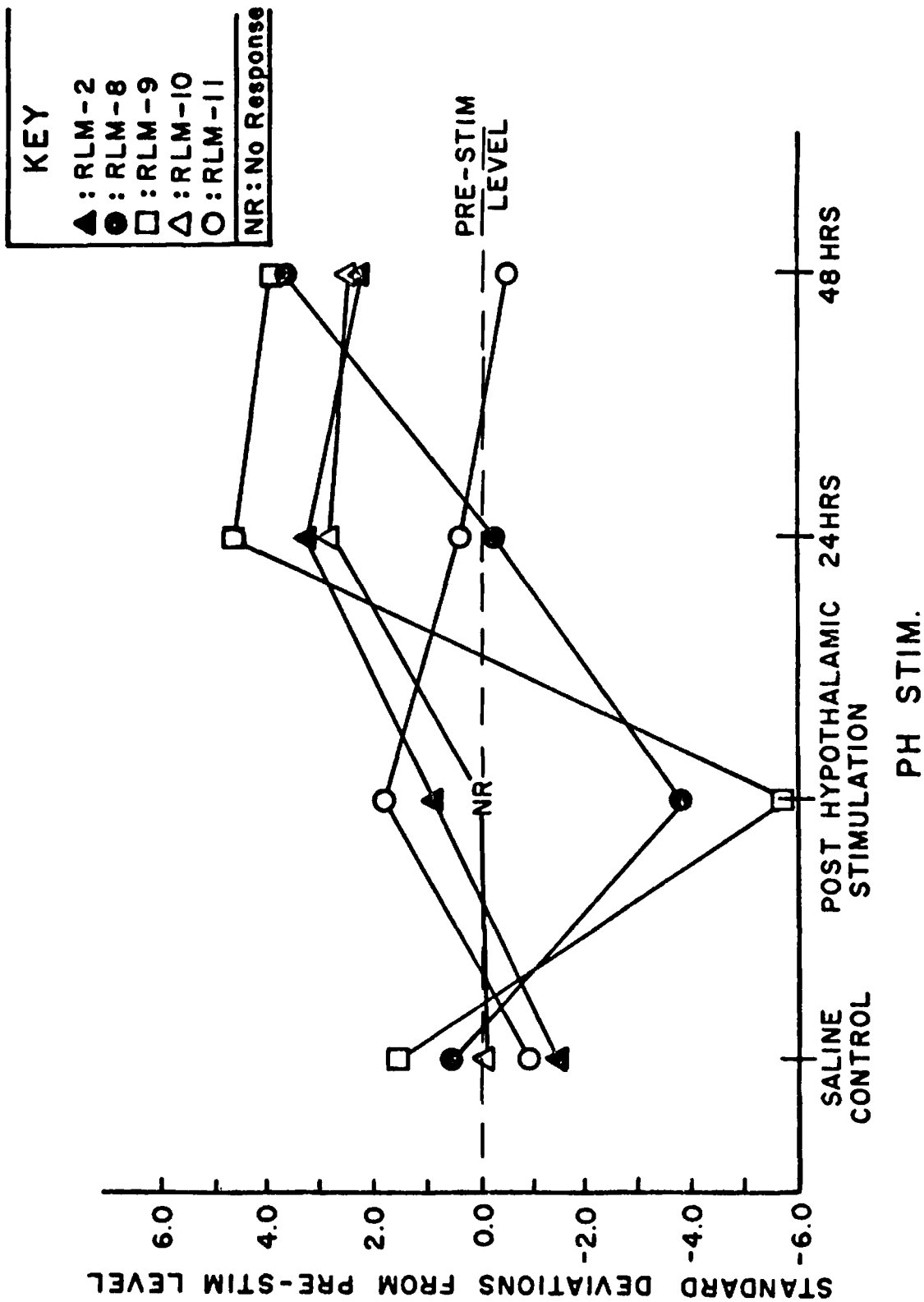

FIGURE 4

PERFORMANCE FOLLOWING POSTERIOR HYPOTHALAMIC STIMULATION

thalamic stimulation were inconsistent and non-significant at all levels.

Electroencephalographic Effects

Analysis of the electroencephalographic (EEG) activity in this experiment was carried out on a hybrid computer system developed in the University of Houston laboratory. A detailed description of the computer can be found elsewhere (Benignus, 1967; Hix, 1969).

The EEG analyses consisted of one of two programs: (1) a continuous band-pass filter (BPF) output through 23%, 1/3 octave filters with center frequencies at 20, 25, 31.5, 40, and 50 c/sec.; and (2) a continuous cross-power function comparing the coherence of power changes in any one EEG band for two brain areas.

The brain structures selected for examination on this visual discrimination task were the lateral geniculate and visual cortices of all animals. In the restricted confines of this chapter, analyses of Cats 9, 10, and 11 are presented as representative of all animals. The figures presented represent the two brain loci under the influence of saline administrations and amphetamine sulfate injections directed to the amygdaloid nuclei. The amygdala was chosen as the stimulation area for EEG analysis due to its pronounced behavioral effects on performance levels in the

visual discrimination task.

Stimulus periods of 10 second durations were halved into 5 second blocks for the band-pass filter output. All BPF figures are composed of the raw EEG output followed by the filtered contribution of each band. The stimulus marker found at the bottom of each figure monitored at 4 c/sec. flashing light. Intrusions into the stimulus pattern indicated a bar-pressing response made by the animal.

Cat 9

Figures 5 and 6 represent the lateral geniculate of Cat 9 under the influence of saline for a typical trial in the 50 trials saline block. Power gains correlated with the evoked potential from the visual stimulus in the lateral geniculate are concentrated across a wide range, including the 25, 31.5, and 40 c/sec. BPF outputs. Administration of amphetamine sulfate to the amygdala slightly desynchronized the activity in the 25 and 31.5 bands in the lateral geniculate in relation to the evoked potential without markedly affecting the 40 c/sec band (see Figures 7 and 8).

Saline conditions in the visual cortex (Figures 9 and 10) were characterized by well-defined bursts of 50 c/sec activity coincident with the evoked potential, and intermittently synchronized power gains coincident with the stimulus in the 40 c/sec. band. Administration of amphetamine resulted in a slight power increase coinciding with the stimulus onset in the 40 c/sec. band. For both the 40 and 50 bands, power

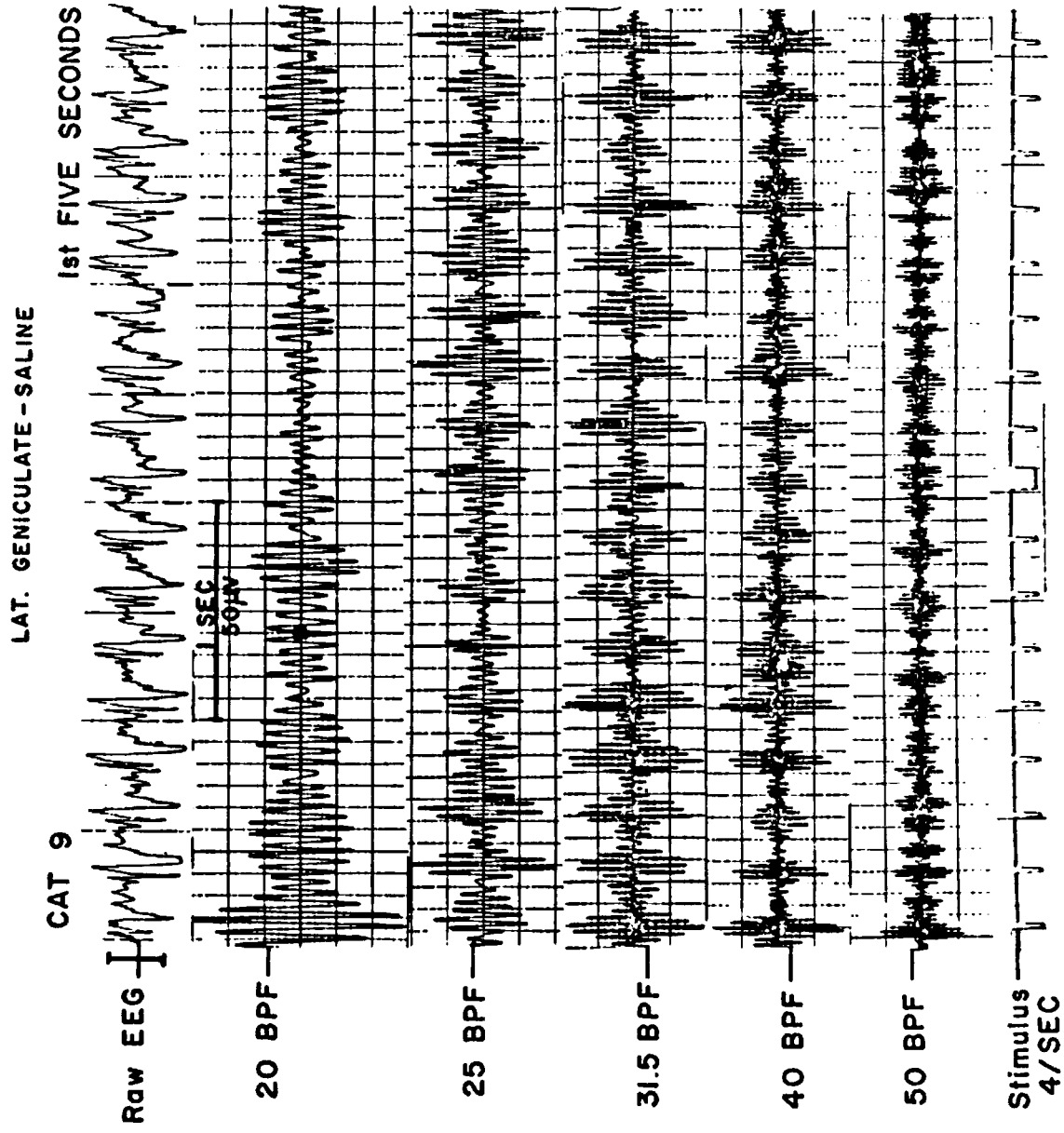

FIGURE 5

BAND-PASS FILTER OUPUTS FOR THE LATERAL GENICULATE OF CAT 9 DURING FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

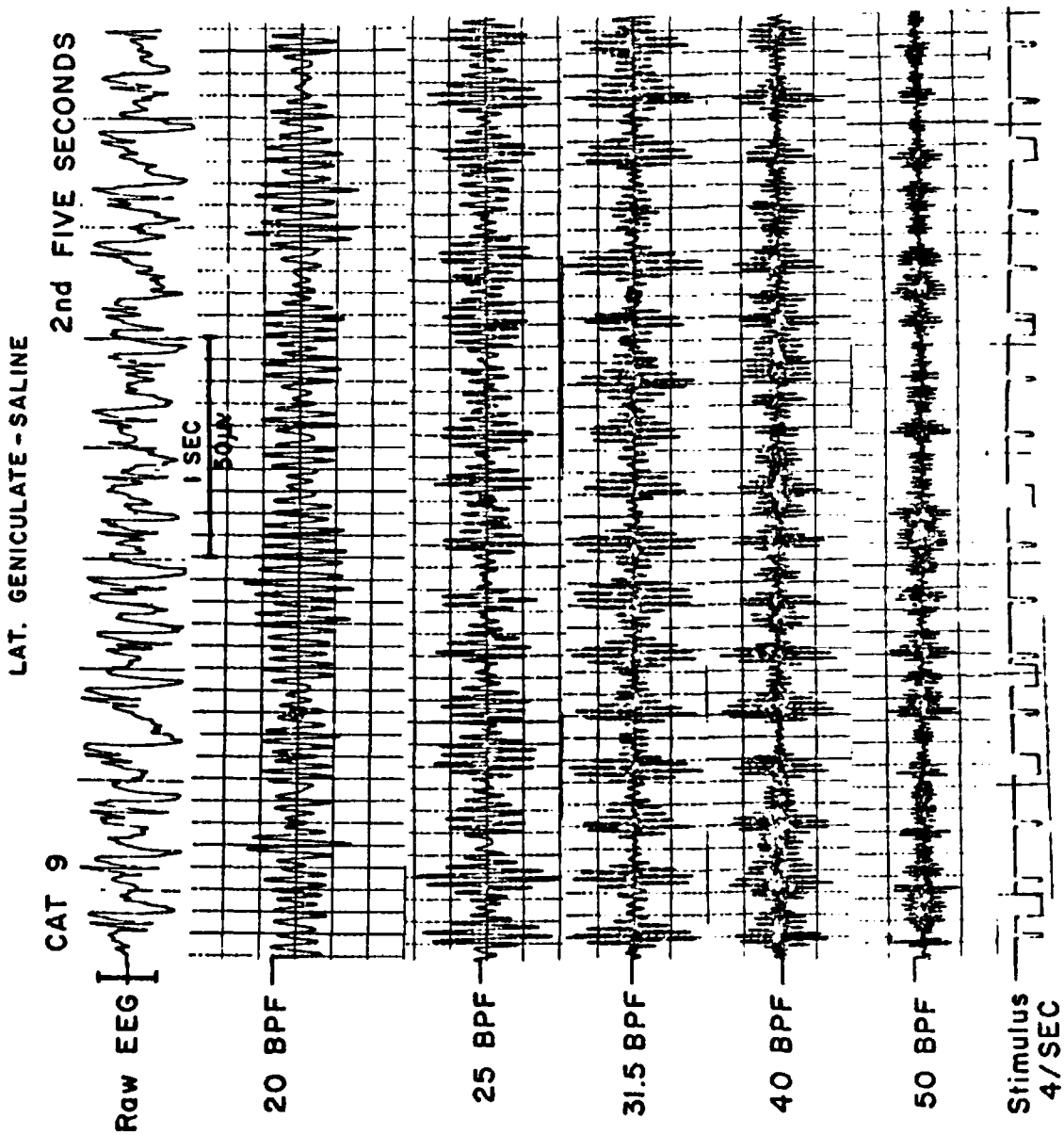

FIGURE 6

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 9 DURING SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

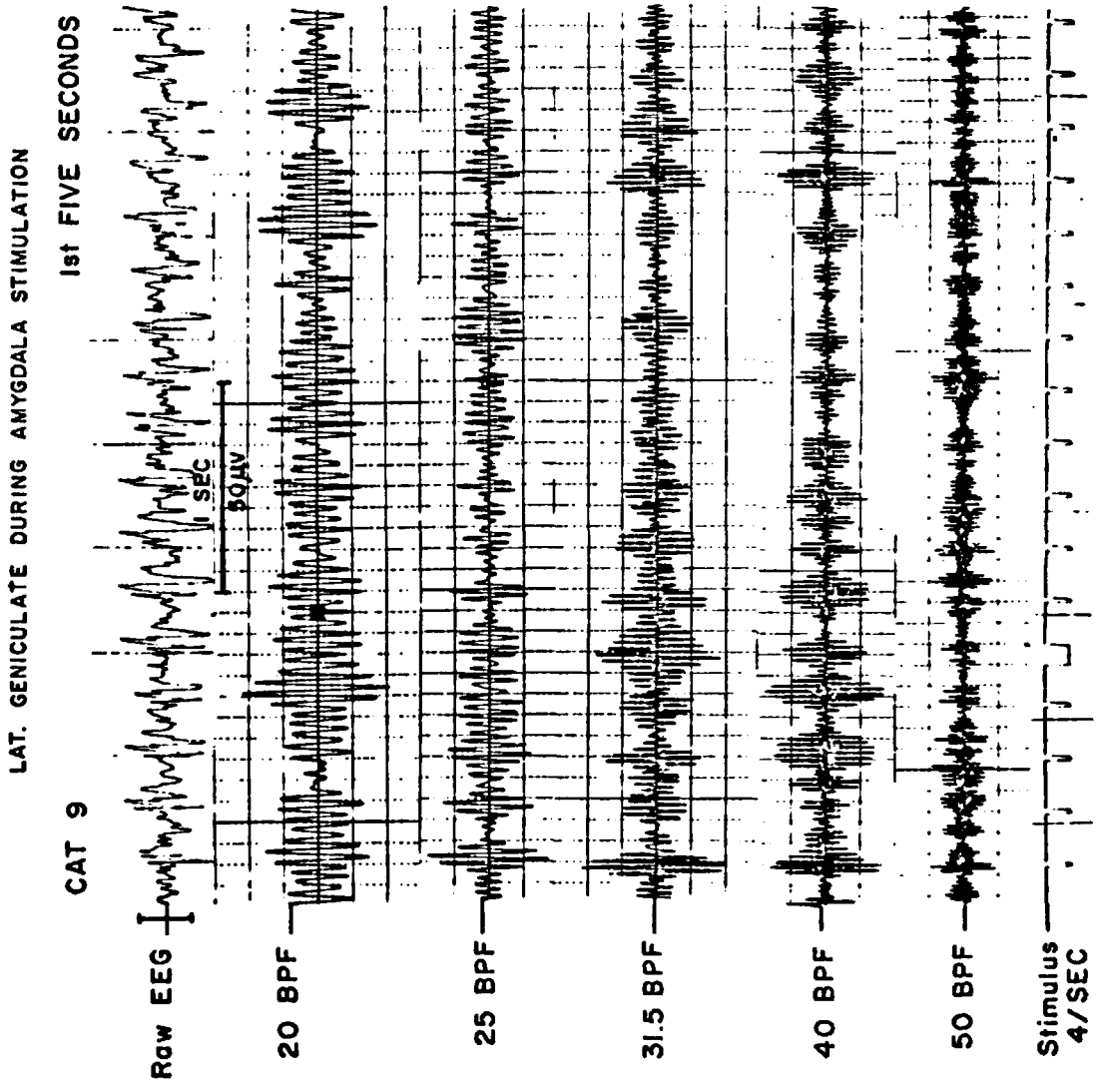

FIGURE 7

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 9 DURING FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

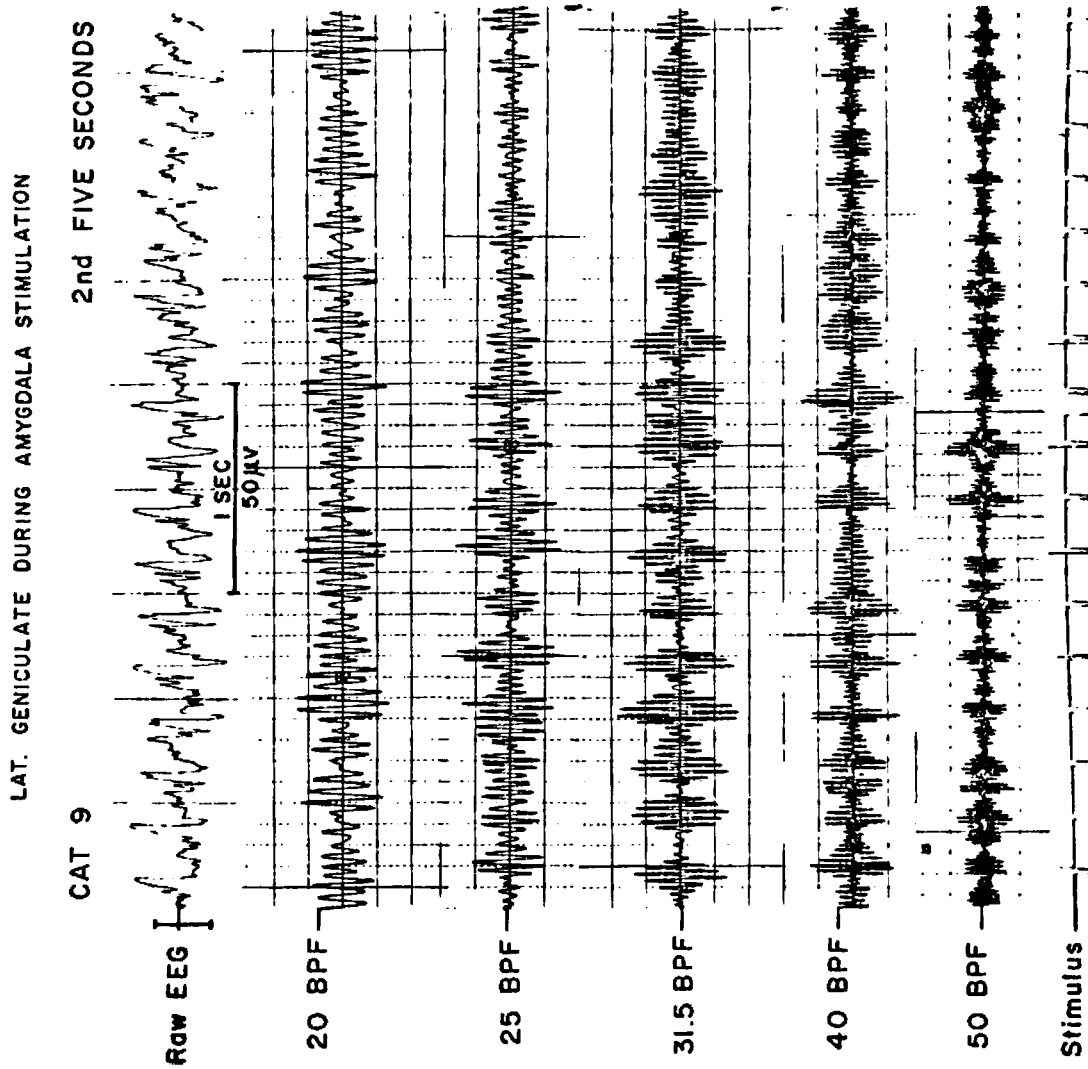

FIGURE 8

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 9 DURING SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

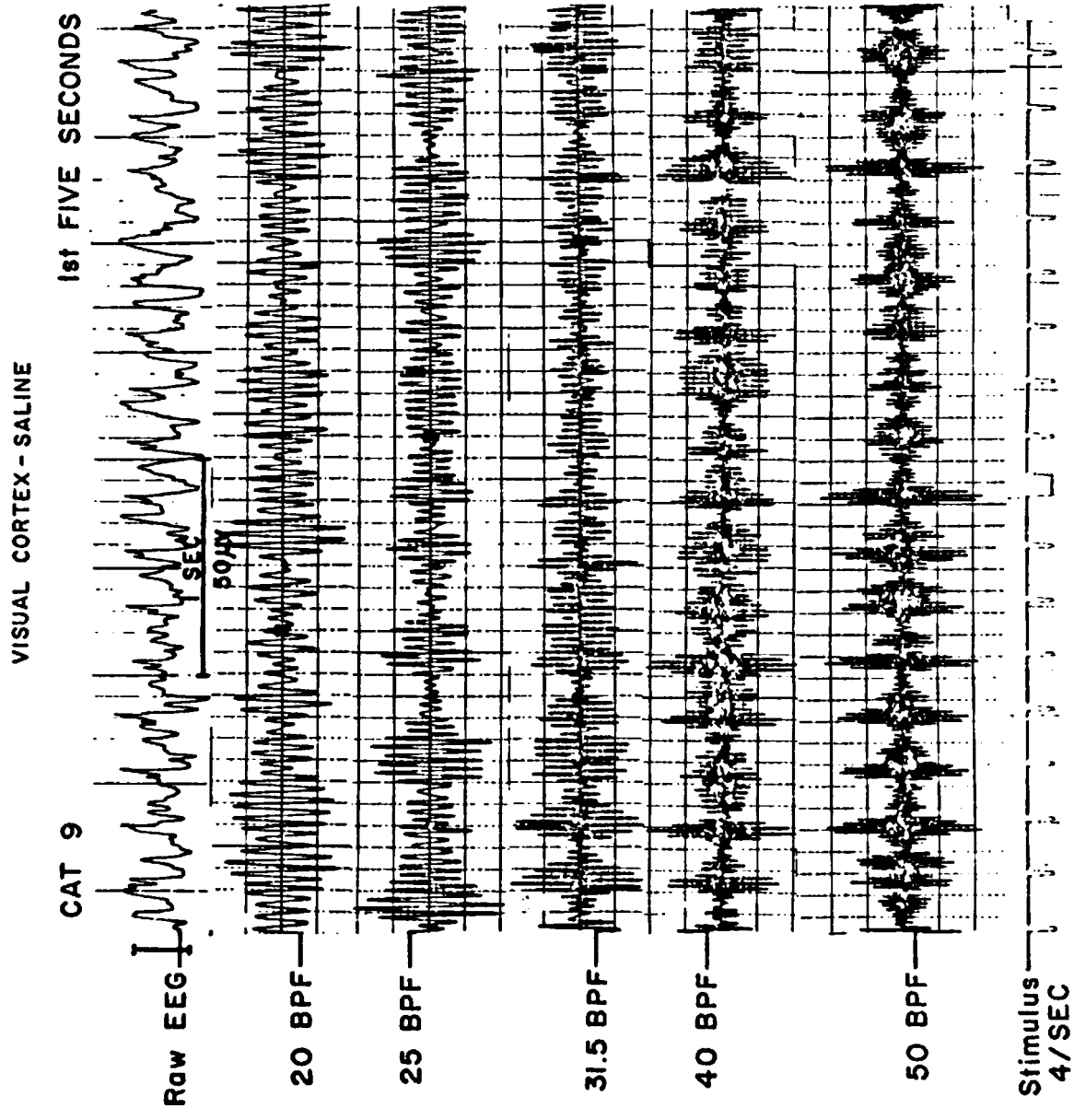

FIGURE 9

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 9 DURING THE FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

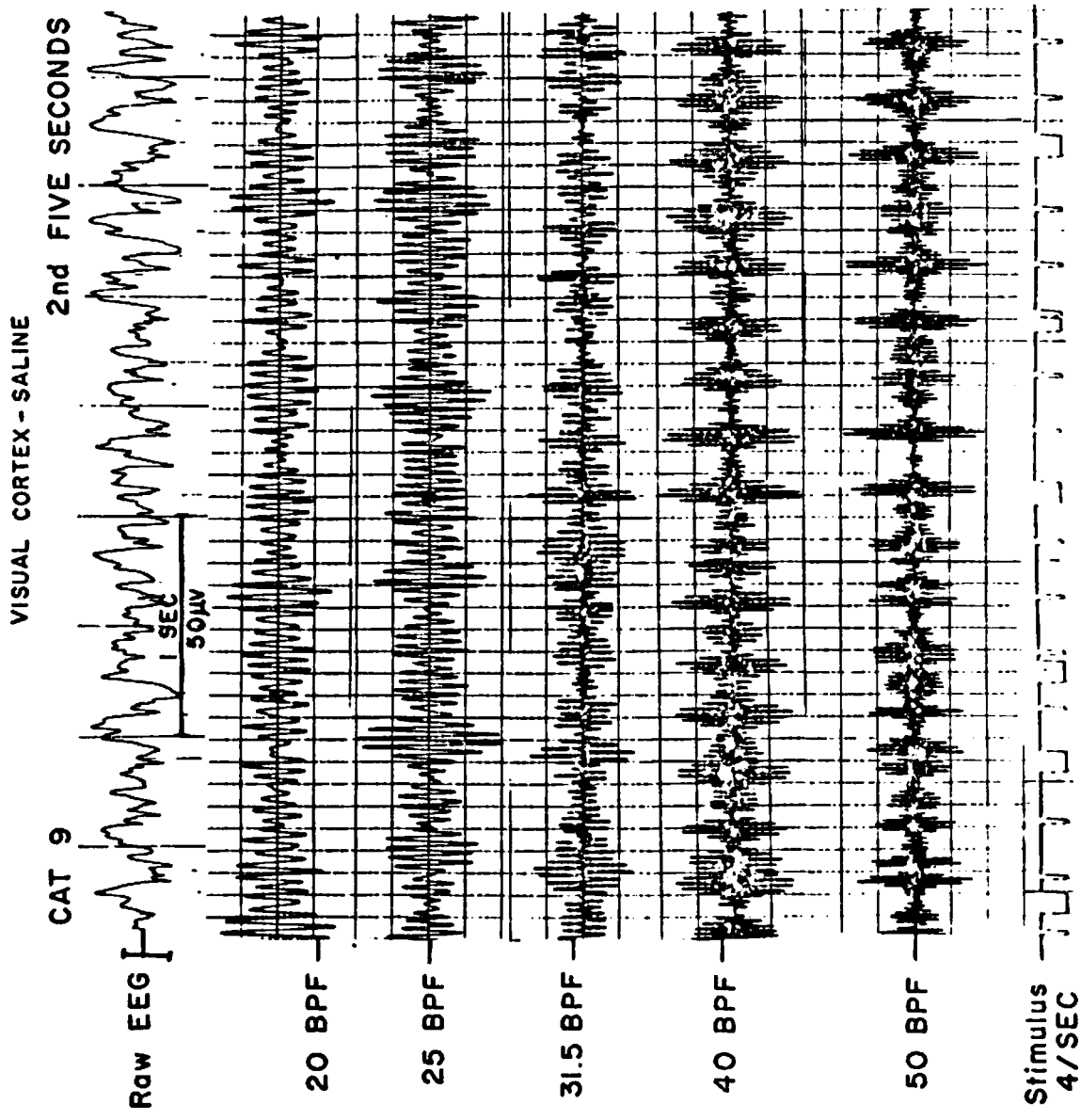

FIGURE 10

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 9 DURING SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

gains became more tightly coordinated with photic driving of the stimulus with large power losses visible between flashes (Figures 11 and 12).

A crosspower function analysis was computed between these two areas for each of the band widths. The highest coherence between these structures occurred in the 40 c/sec. band, which is represented by Figure 13. Presented in this figure was the crosspower available in the ten seconds prior to the onset of the 10 second stimulus period, as well as the SD period itself. The trials selected were representative of EEG patterns present in amygdala, saline and baseline trial blocks. As can be seen, virtually no coherence of power was present in the pre-stimulus period; however, a high coherence of crosspower was visible at the onset of the stimulus for the amygdala stimulation condition. This coherence pattern was not present in the saline and baseline conditions.

Cat 10

Figures 14 and 15 represent the EEG band-pass filter outputs from the lateral geniculate during saline administration for Cat 10. Power gains coincident with photic driving were observed in 25, 31.5, 40, and 50 c/sec. bands with a noticeable dominance in the 31.5 band-pass filter output. Administration of amphetamine sulfate in the amygdala produced results seen in Figures 16, and 17. The activity in the 50 c/sec. band diminished in relation to the photic stimulus. However, a clear shift occurred in the 31.5 and 40

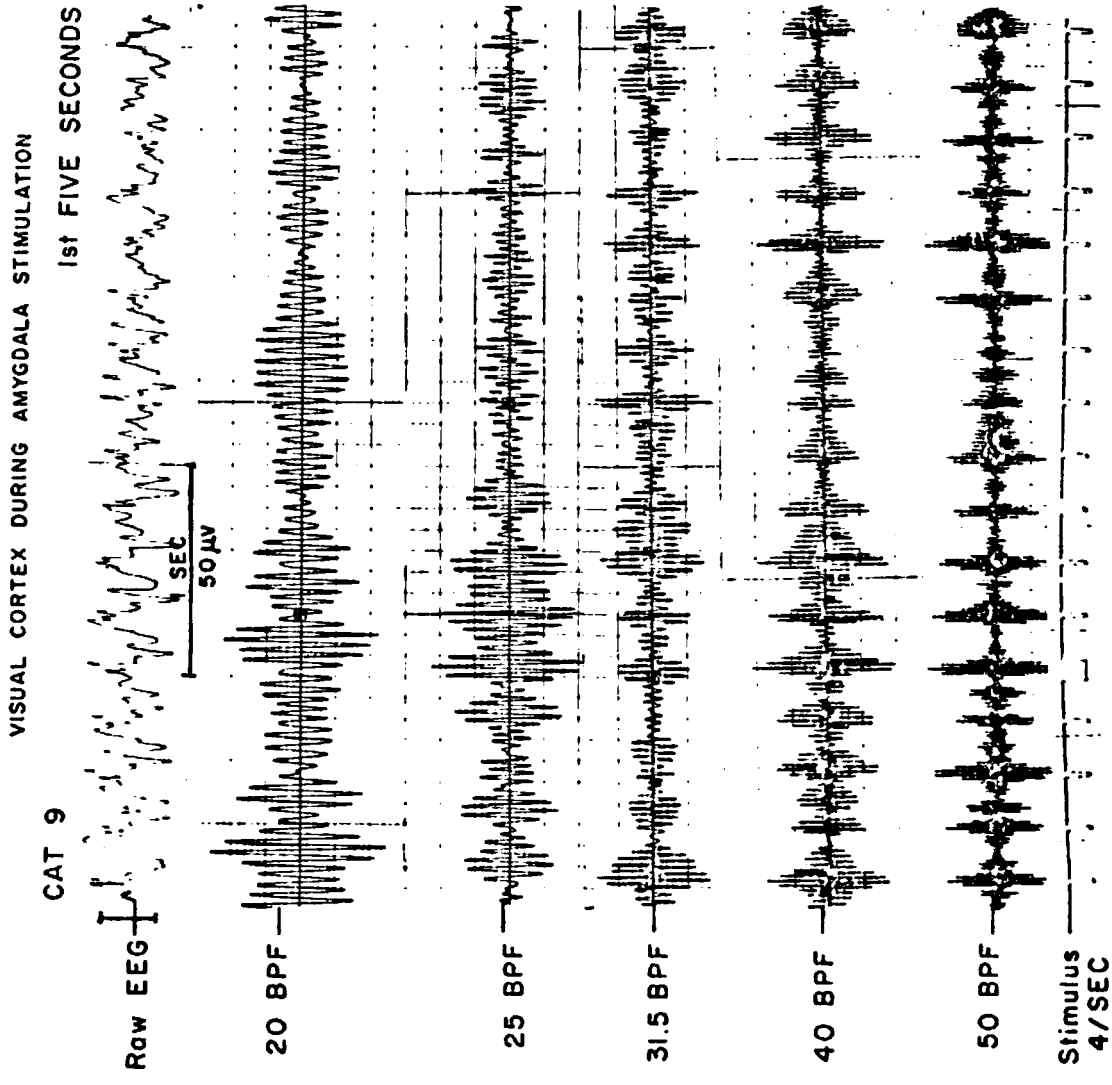

FIGURE 11

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 9 DURING FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

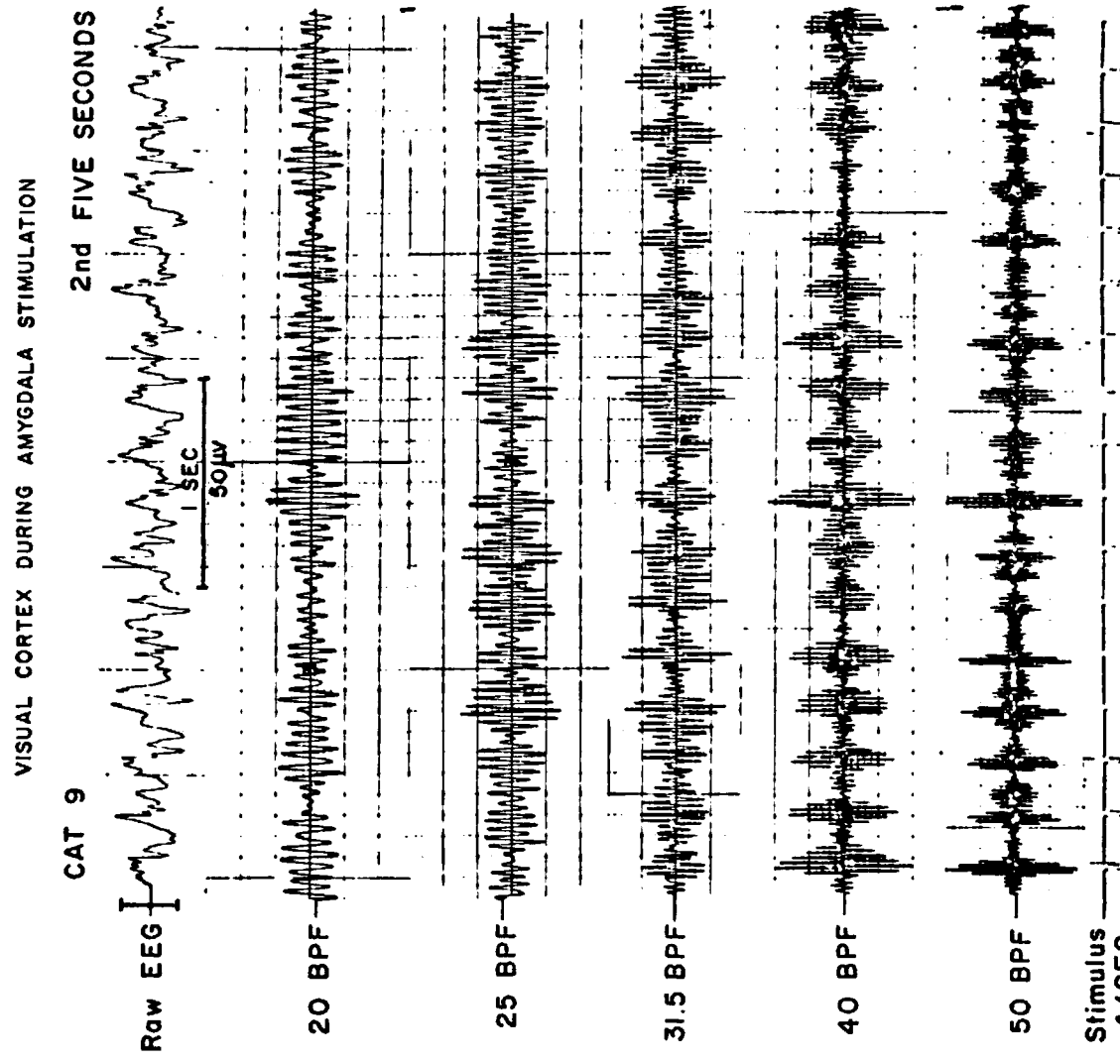

FIGURE 12
BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 9 DURING SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

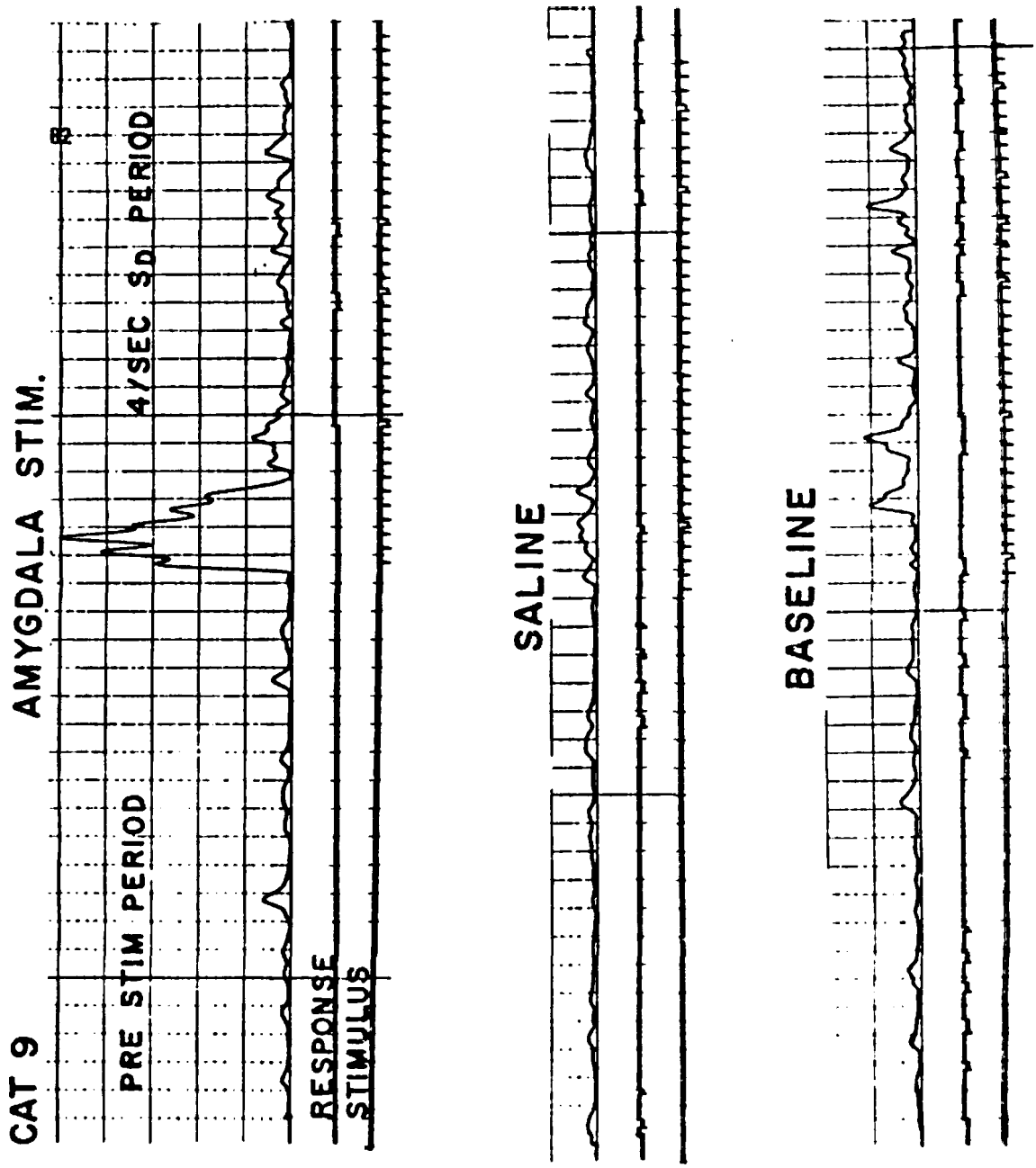

FIGURE 13

CROSSPOWER FUNCTION BETWEEN LATERAL GENICULATE AND VISUAL CORTEX OF CAT 9 DURING AMYGDALA STIMULATION, SALINE, AND BASELINE CONDITIONS

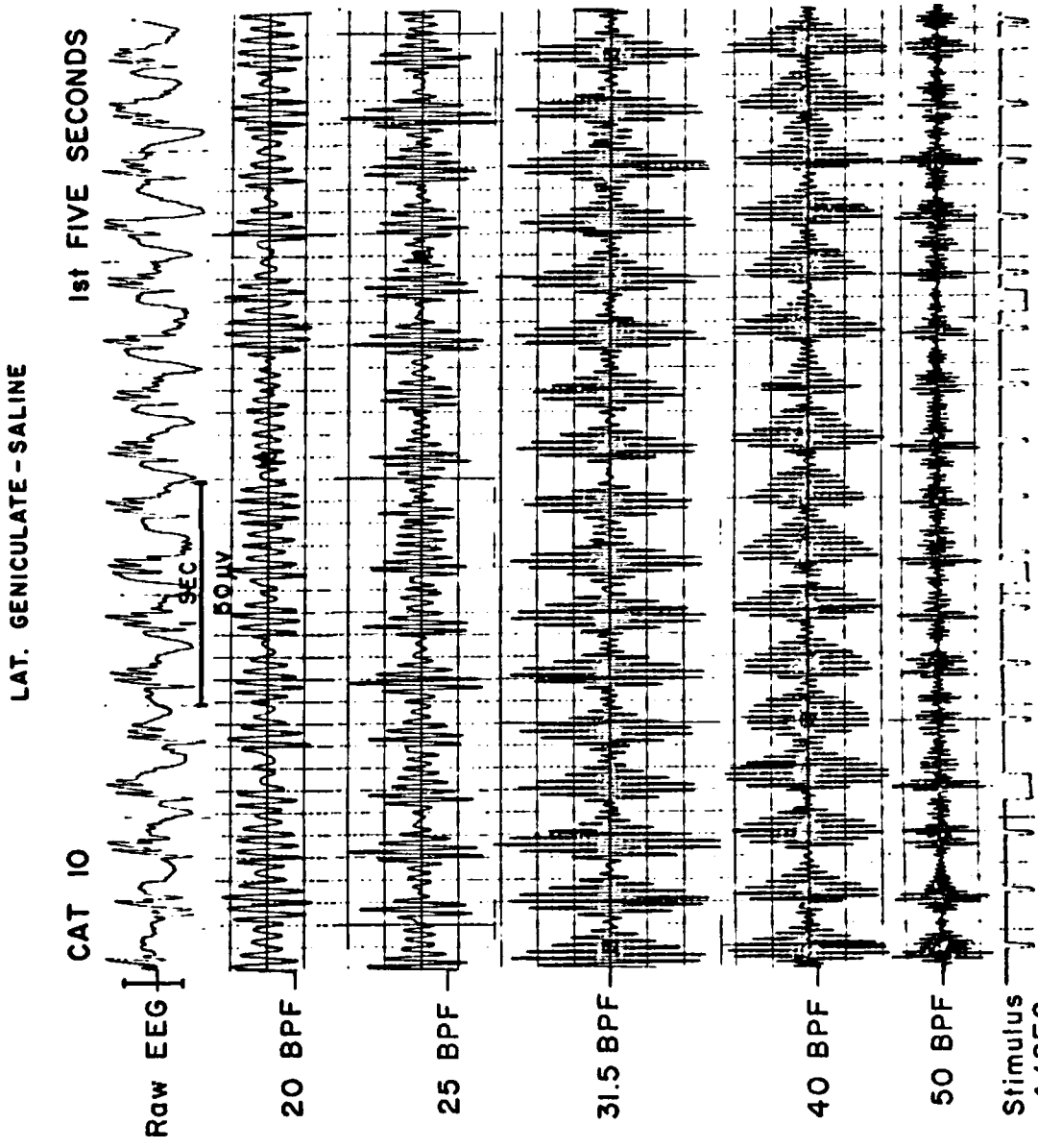

FIGURE 14

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 10 DURING THE FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

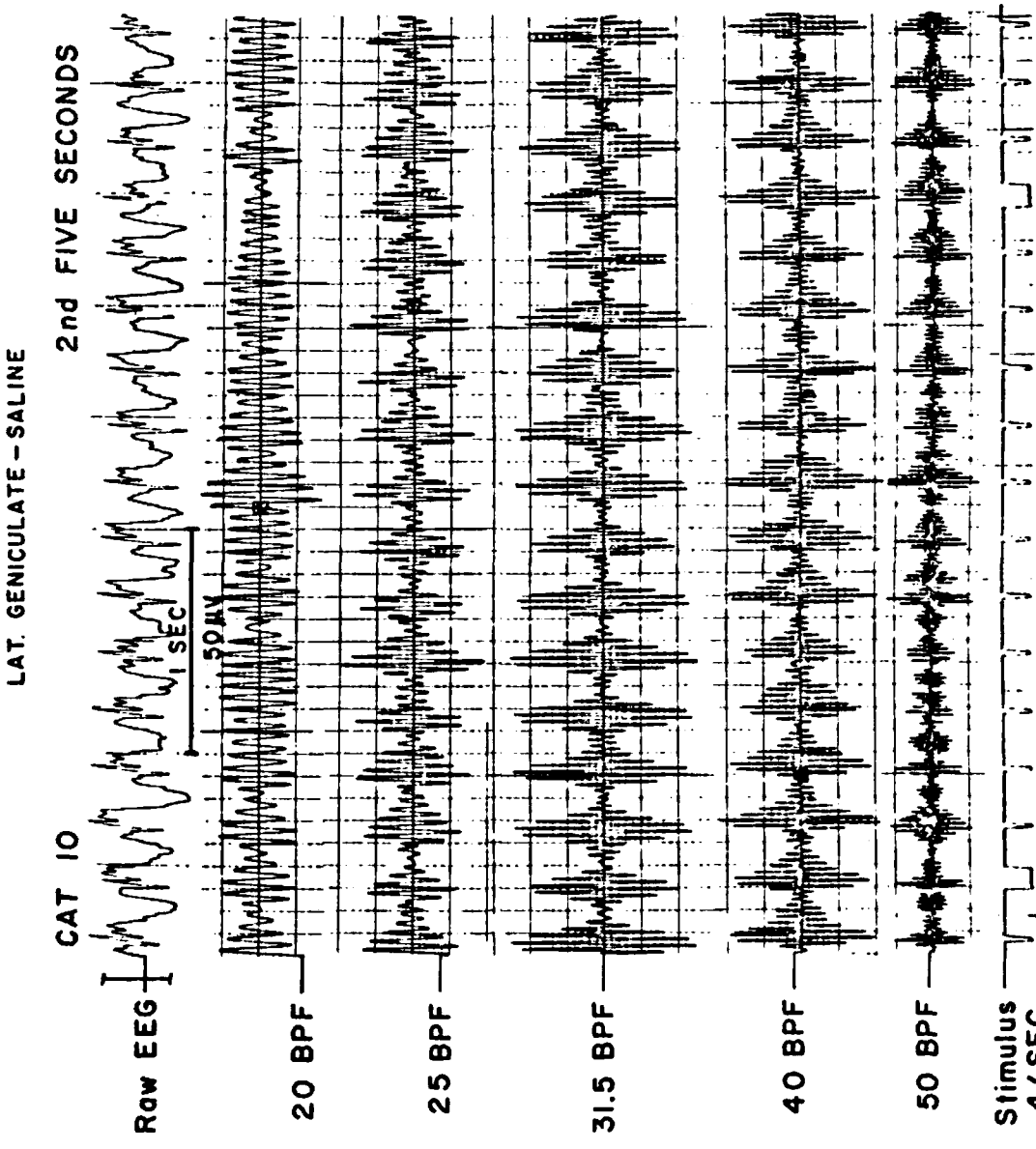

FIGURE 15

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 10 DURING THE SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

c/sec. bands. The 40 band clearly became the dominant frequency range, displaying massive synchronized power gains, clearly demarcated by the stimulus flashes.

The visual cortex under saline stimulation is represented by Figures 18 and 19. No clear pattern of power gain was visible in any of the five frequency bands. However, administration of amphetamine produced changes in the 40 and 50 c/sec. BPF outputs. A noticeable power gain was seen with photic driving in these two frequency ranges (Figures 20 and 21).

Crosspower analysis between the lateral geniculate and visual cortex of Cat 10 is displayed in Figure 22. Again all three conditions were represented by a single trial, characteristic of its particular experimental condition. Concentration of coherence of crosspower was absent in the pre-stimulus period for all three conditions; however, the stimulus period for amphetamine administration was characterized by a very high crosspower value at stimulus onset. This characteristic was absent in saline and baseline conditions.

An additional phenemenon was observed in both visual cortex and lateral geniculate for this animal. The saline 20 c/sec. band (Figures 14,15,18, and 19) displayed little variability in power output; however, under the influence of amphetamine stimulation, distinct power increases were visible following responses (Figures 16, 17,20, and 21).

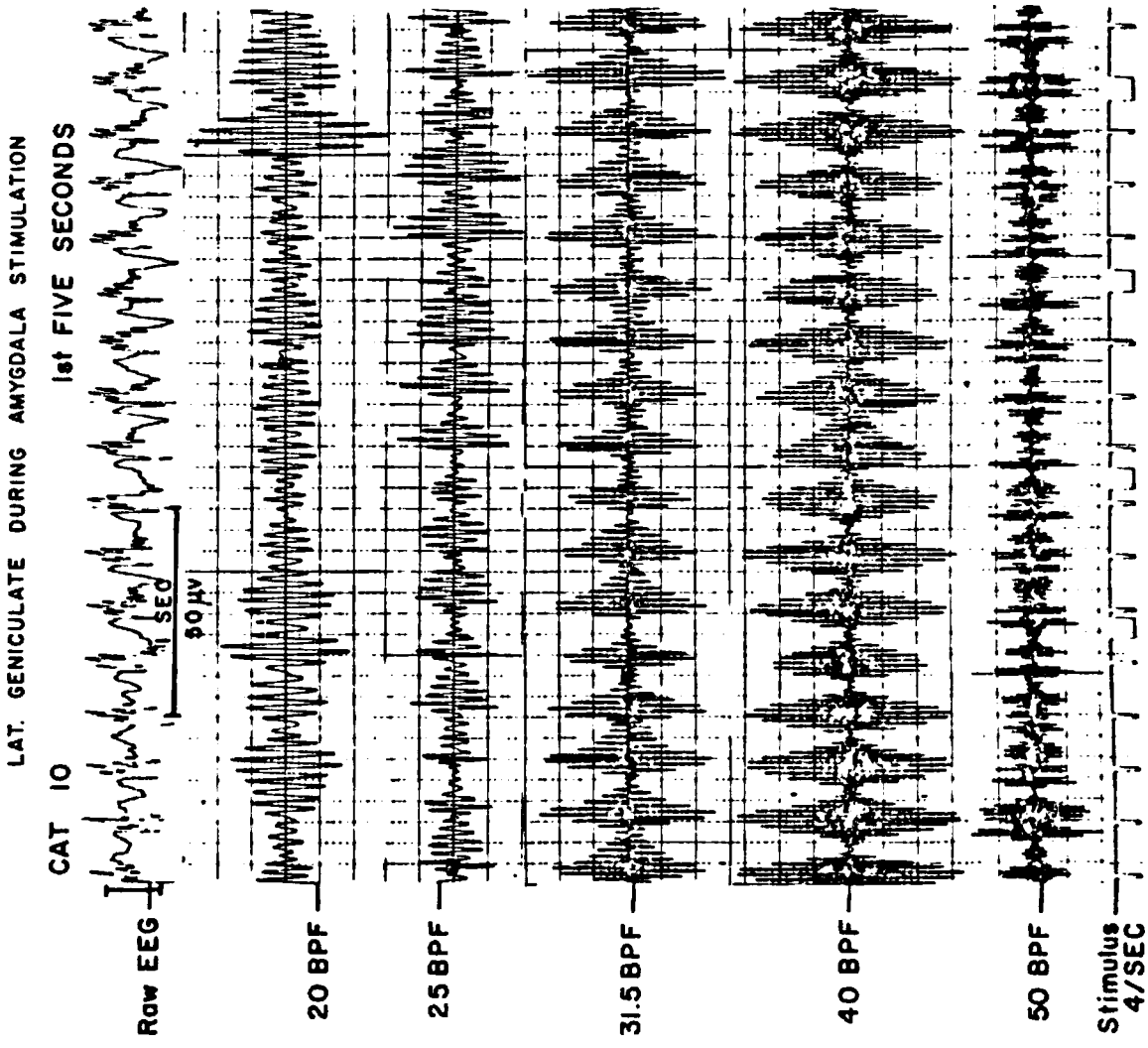

FIGURE 16

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 10 DURING THE FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

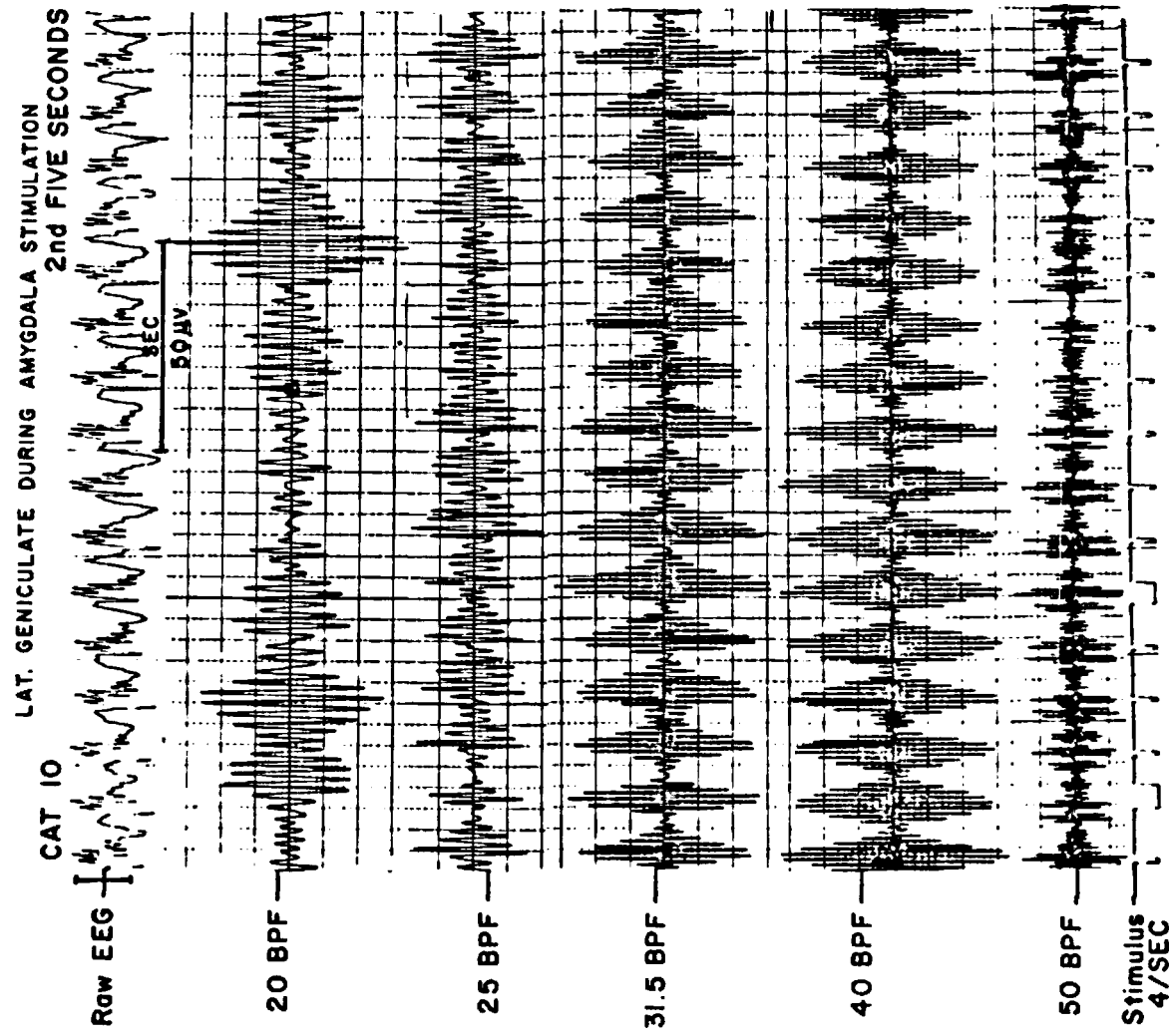

FIGURE 17

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 10 DURING THE SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

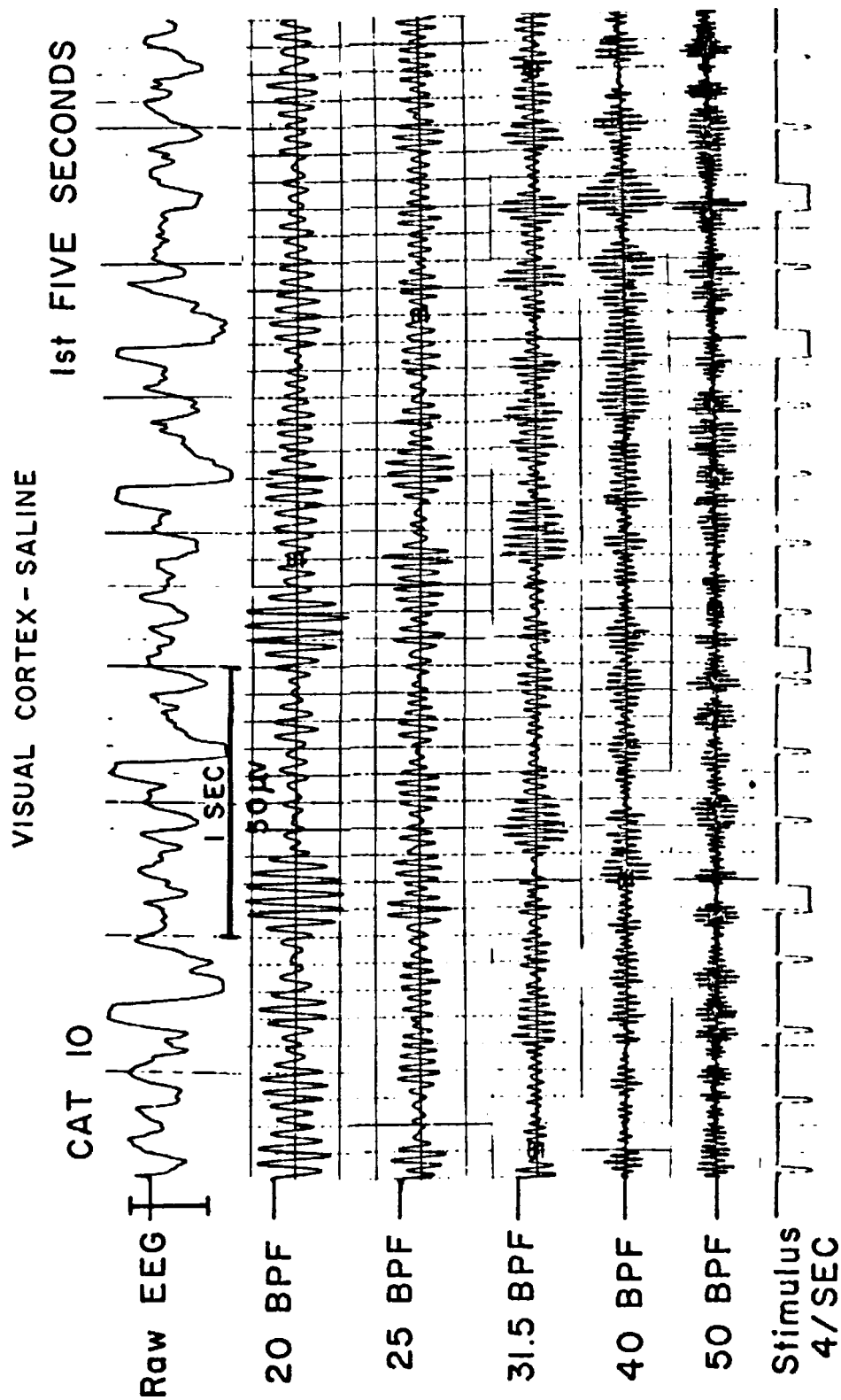

FIGURE 18

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 10 DURING THE FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

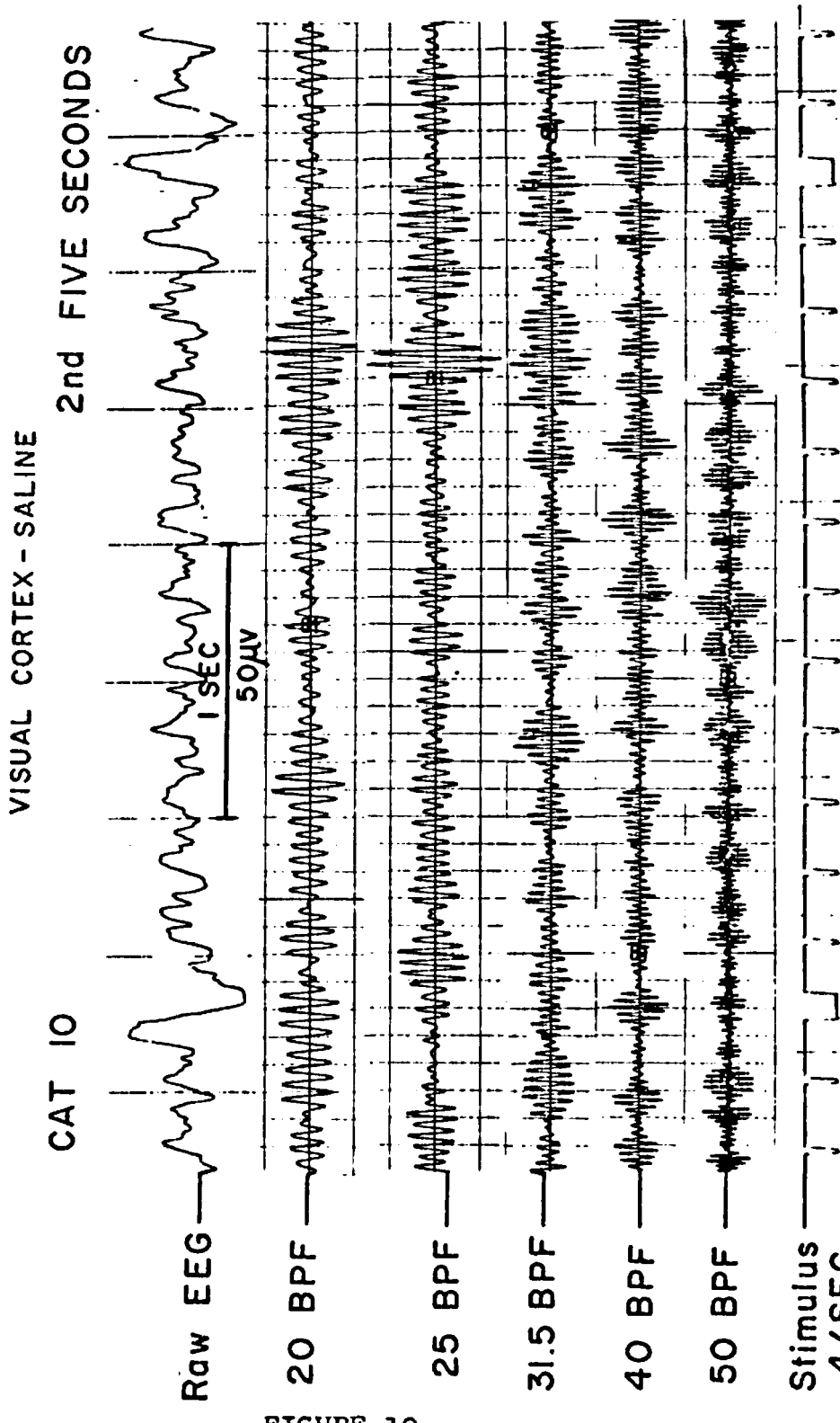

FIGURE 19

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 10 DURING THE SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

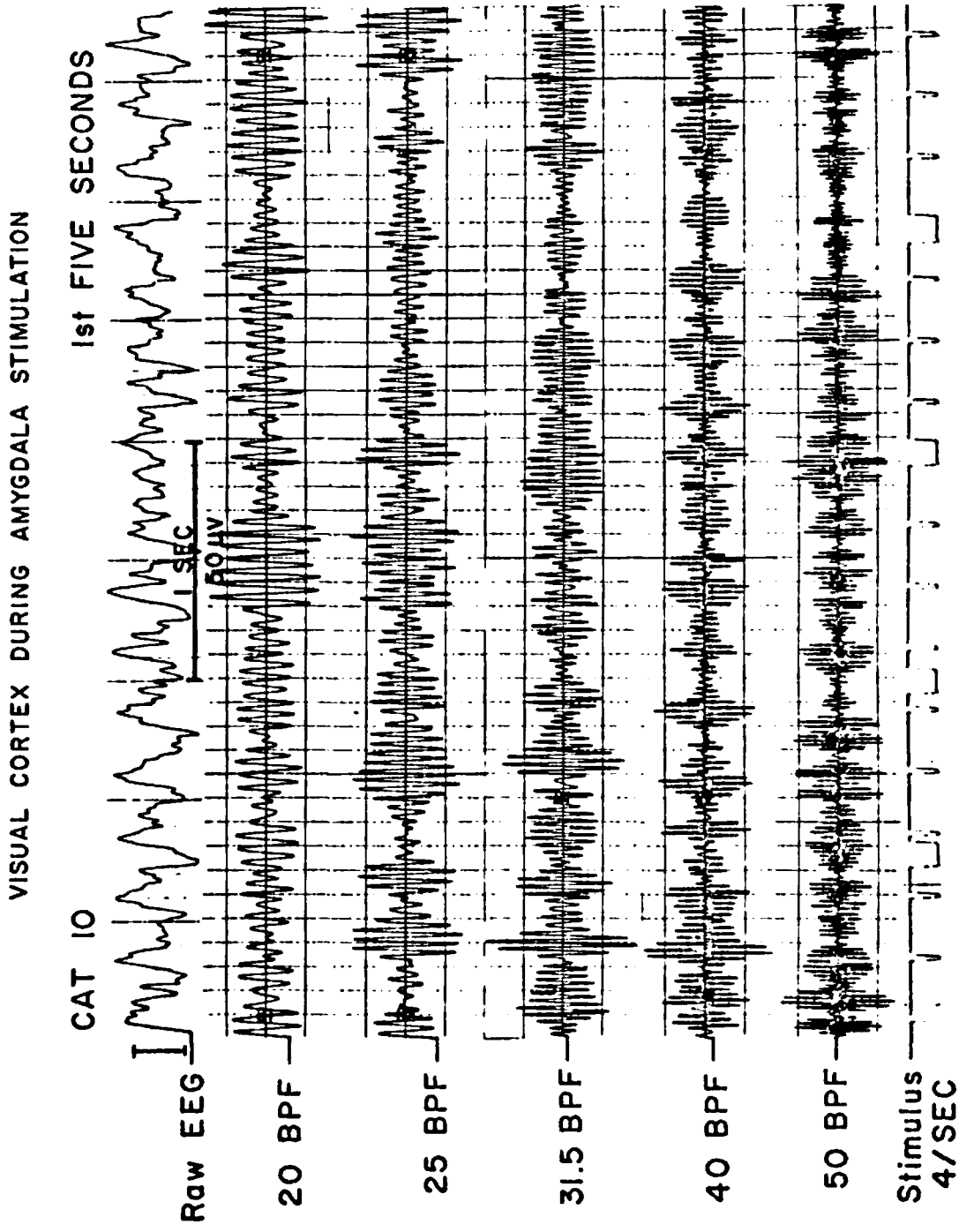

FIGURE 20

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 10 DURING THE FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERICD

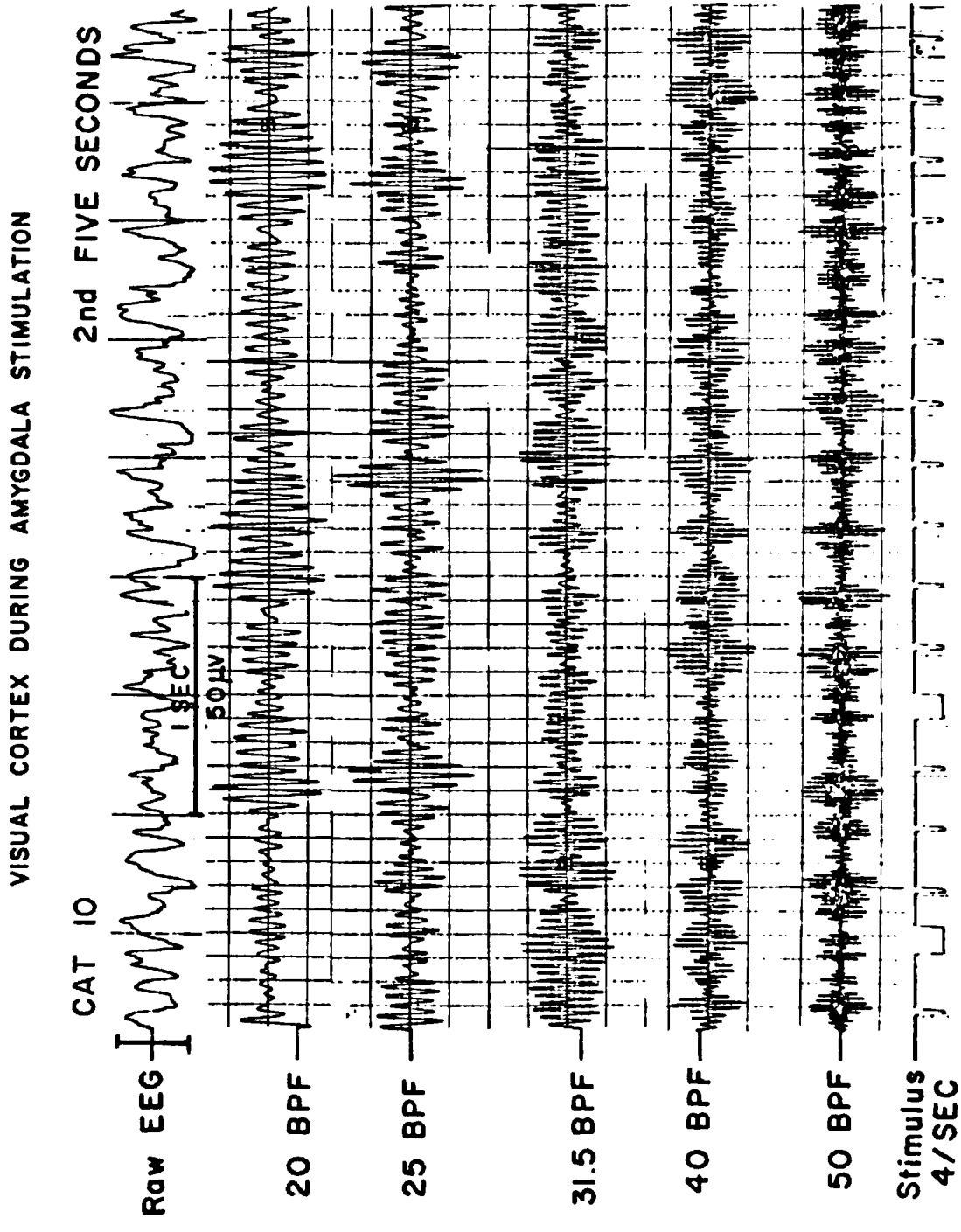

FIGURE 21
BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 10 DURING THE SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

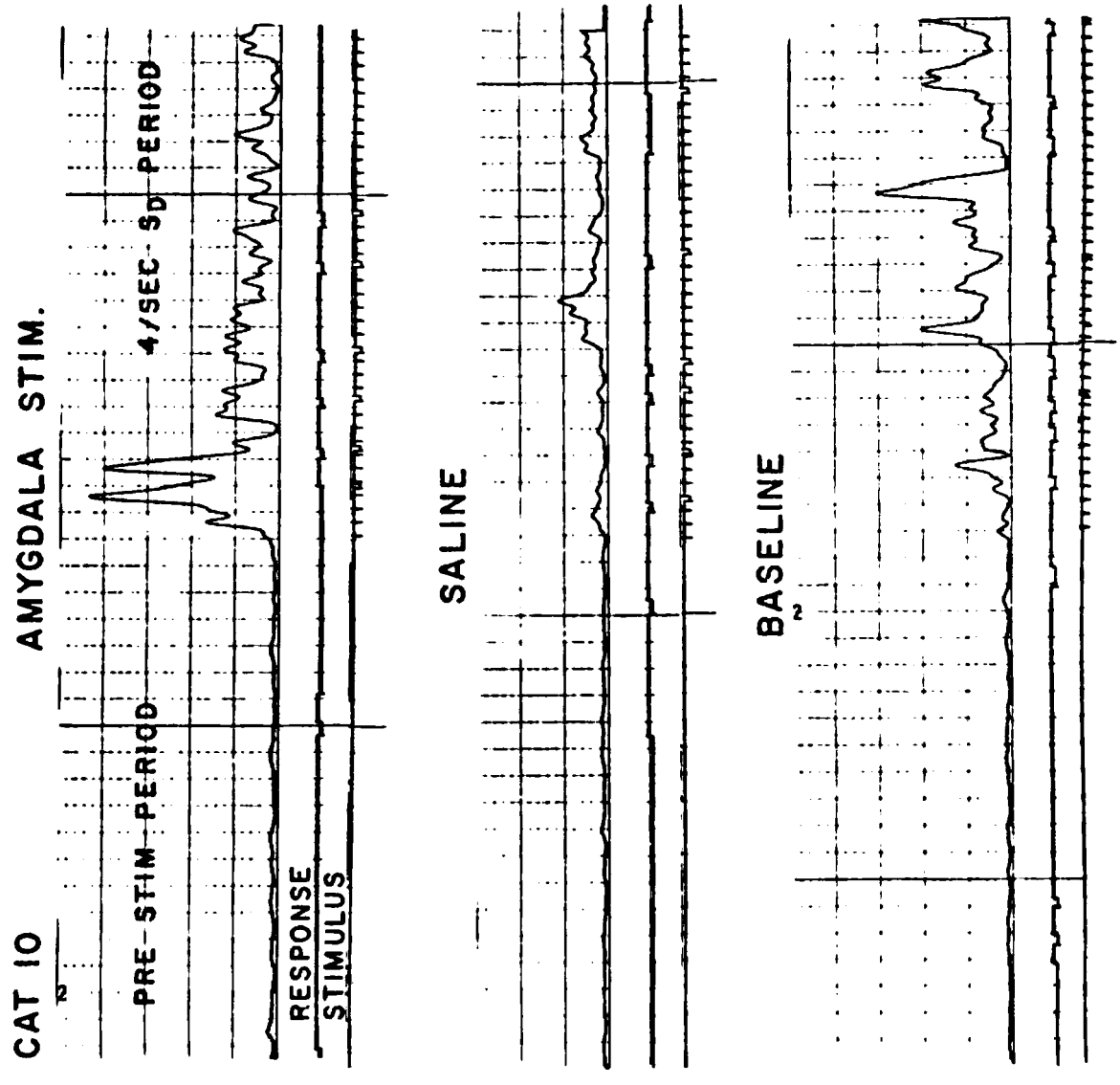

FIGURE 22

CROSSPOWER FUNCTION BETWEEN LATERAL GENICULATE AND VISUAL CORTEX OF CAT 10 DURING AMYGDALA STIMULATION, SALINE, AND BASELINE CONDITIONS

Cat 11

The lateral geniculate of Cat 11 during saline was characterized by a dominance of power gains in the 31.5 and 40 c/sec. bands with some increase visible in the 50 c/sec. band (Figures 23 and 24). Following administration of amphetamine sulfate, a power shift was observed (Figures 25 and 26). The 40 c/sec. band-pass filter became dominant displaying large power outputs, coincident with the photic flashes. The 50 c/sec. frequency band also appeared more synchronized with the photic stimulus.

The visual cortex during the saline condition showed evidence of a dominant power gain in the 50 c/sec. band coincident with photic driving (Figures 27 and 28). An inconsistent pattern of power bursts with the stimulus were observed in the 40 c/sec. frequency. Following administration of the amphetamine sulfate to the amygdala, the width (time span) of the power increases in the 50 c/sec. band narrowed to more closely approximate the temporal patterns of the stimulus. The 40 c/sec. band increased its power and displayed the same "sharpening" to the stimulus as was seen in the 50 c/sec. range (Figures 29 and 30).

The 40 c/sec. crosspower function between the lateral geniculate and visual cortex of Cat 11 (Figure 31) characterized the relationship seen in Cats 9 and 10. Again representative trials were selected for each of the three conditions indicated. High coherence was observed at the onset of

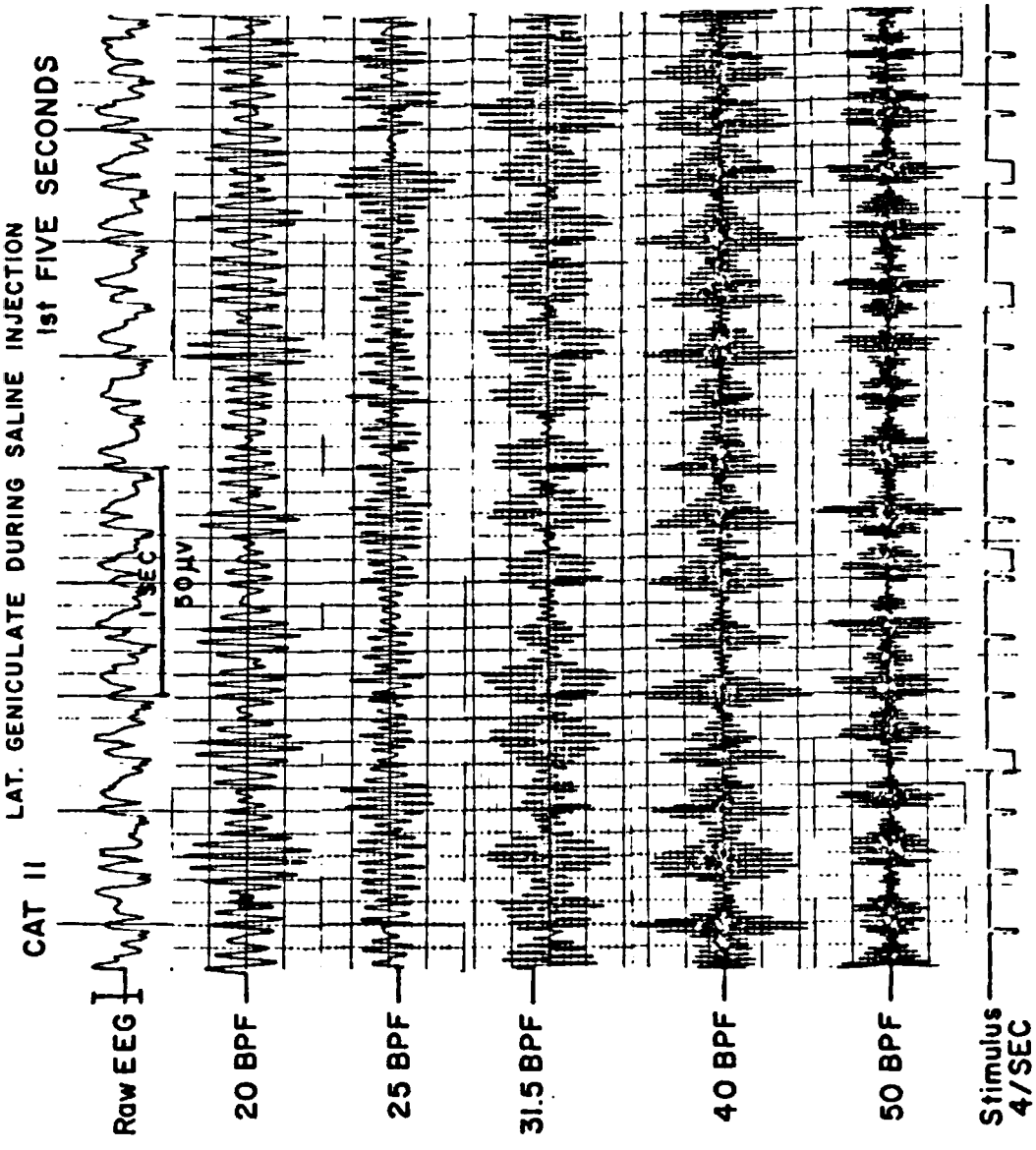

FIGURE 23

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 11 DURING THE FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

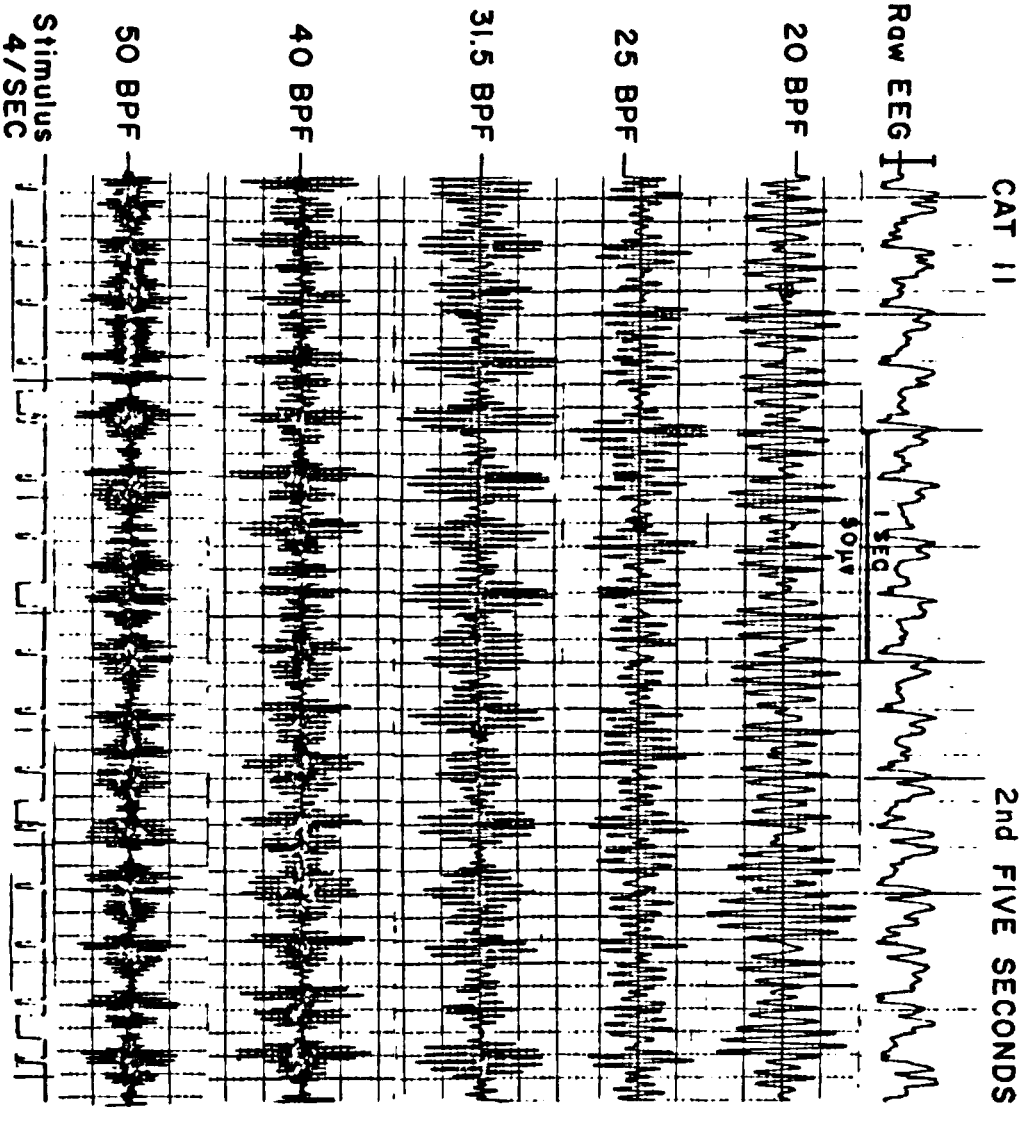

FIGURE 24

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 11 DURING THE SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

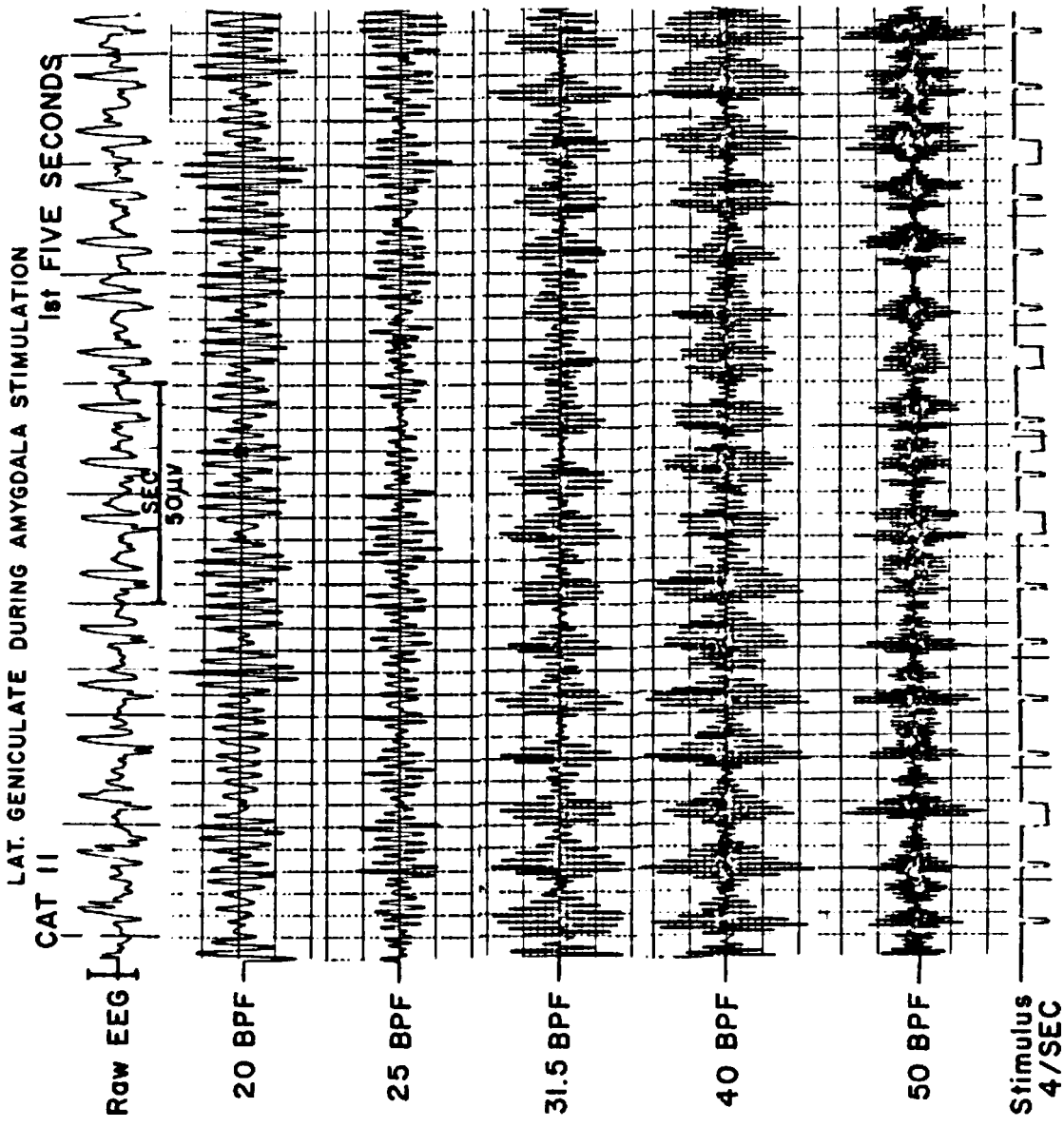

FIGURE 25

BAND-PASS FILTER OUPUTS FOR THE LATERAL GENICULATE OF CAT 11 DURING THE FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

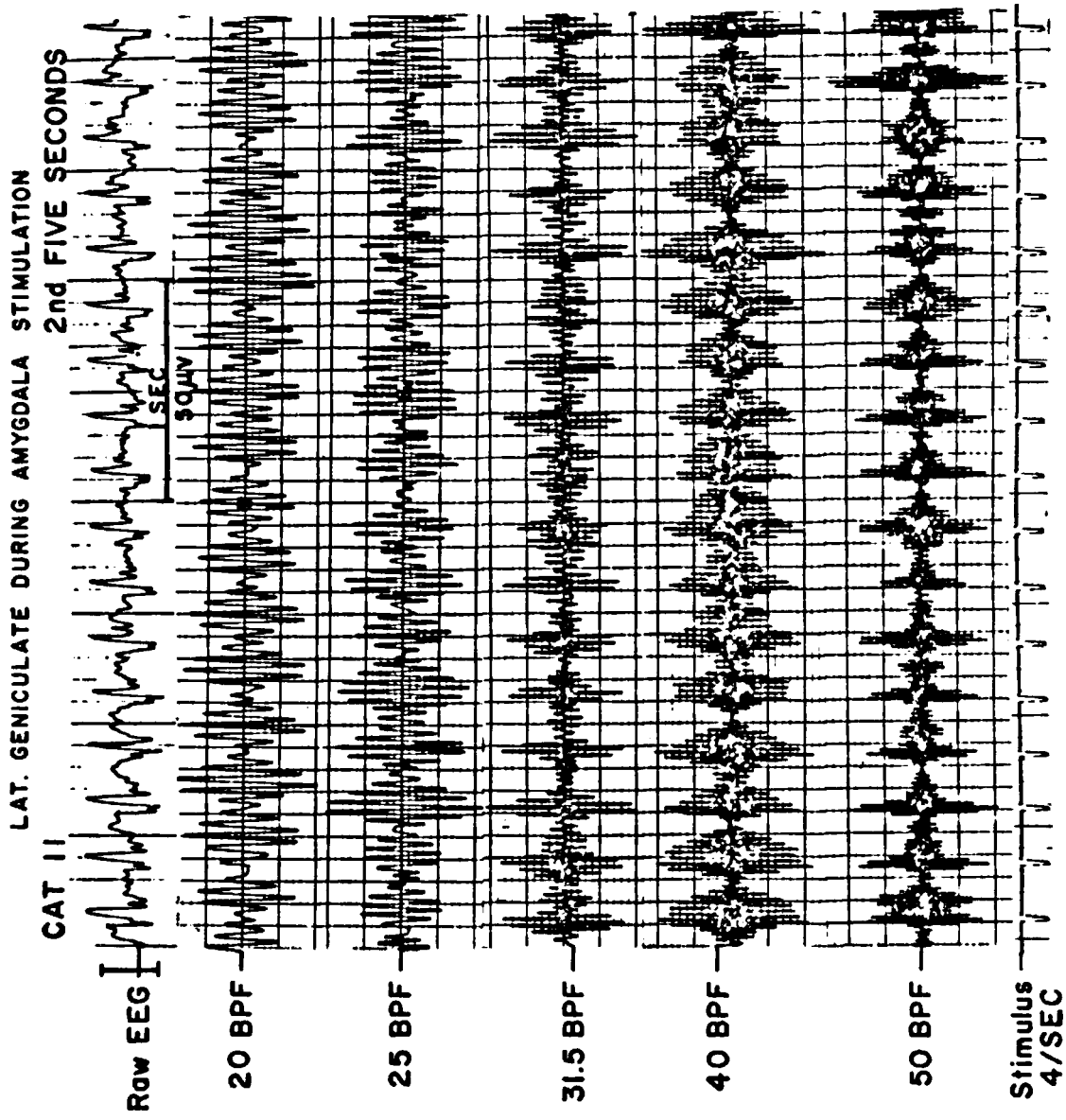

FIGURE 26

BAND-PASS FILTER OUTPUTS FOR THE LATERAL GENICULATE OF CAT 11 DURING THE SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

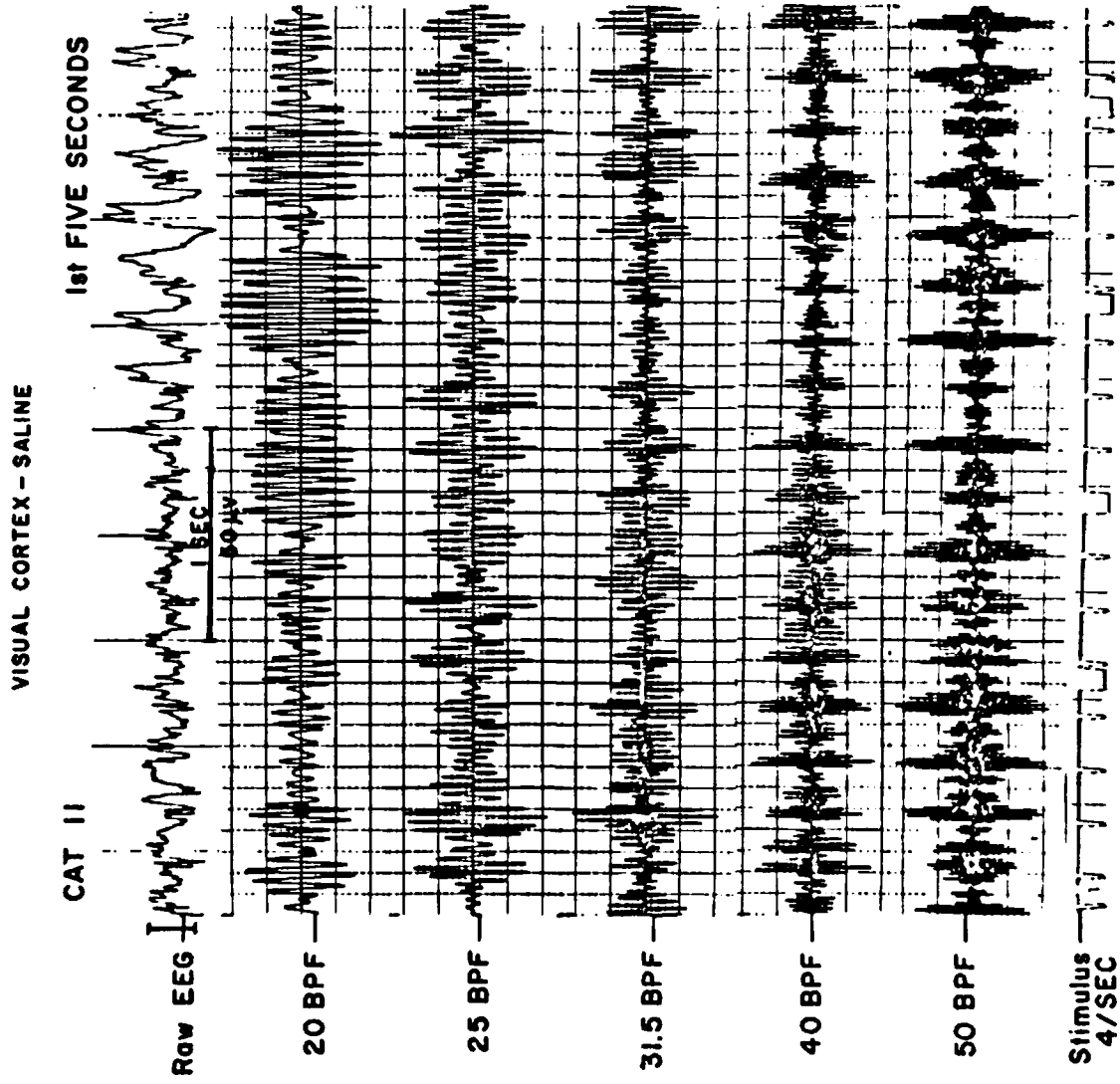

FIGURE 27

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 11 DURING THE FIRST FIVE SECONDS OF SALINE STIMULUS PERIOD

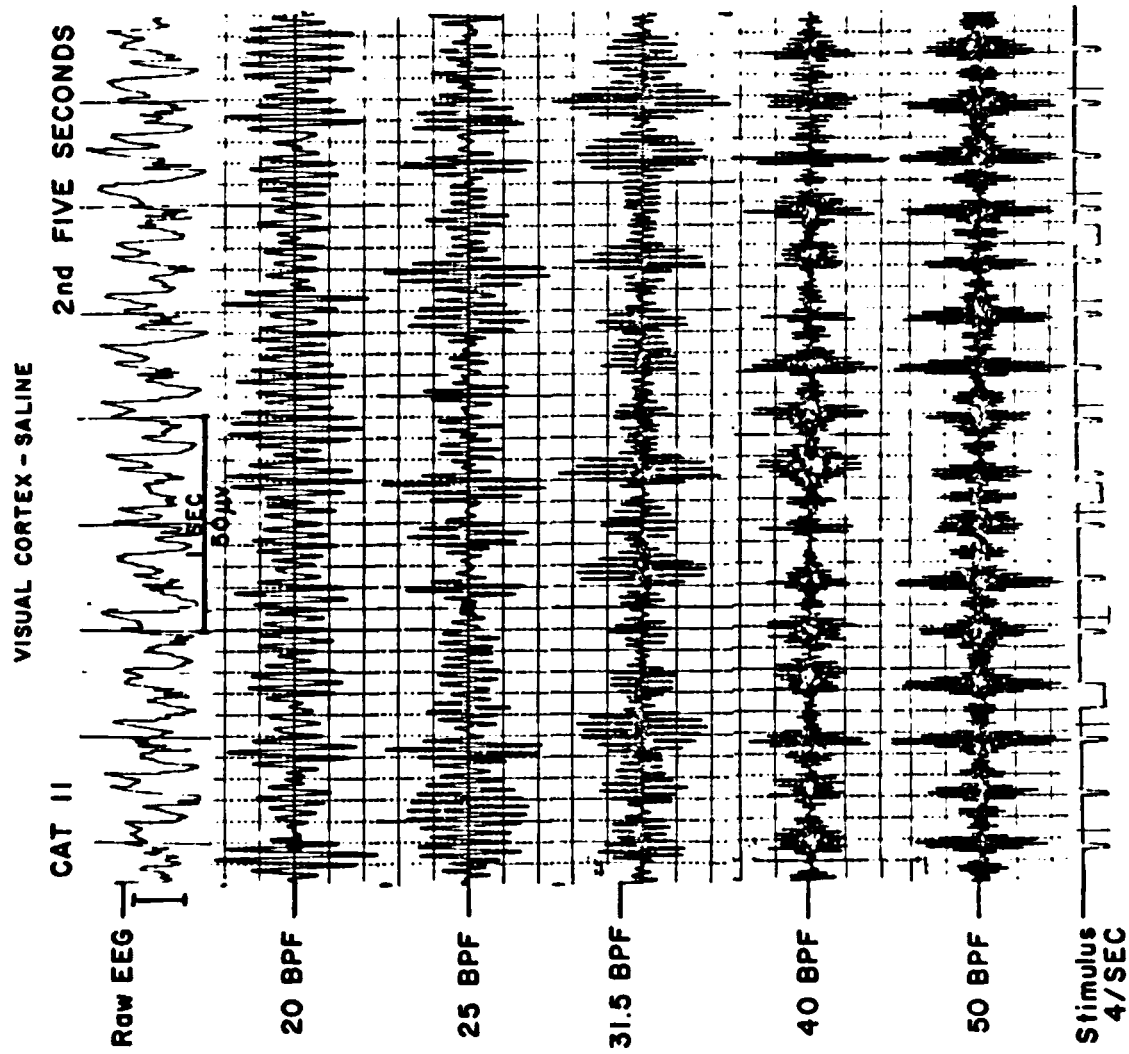

FIGURE 28

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 11 DURING THE SECOND FIVE SECONDS OF SALINE STIMULUS PERIOD

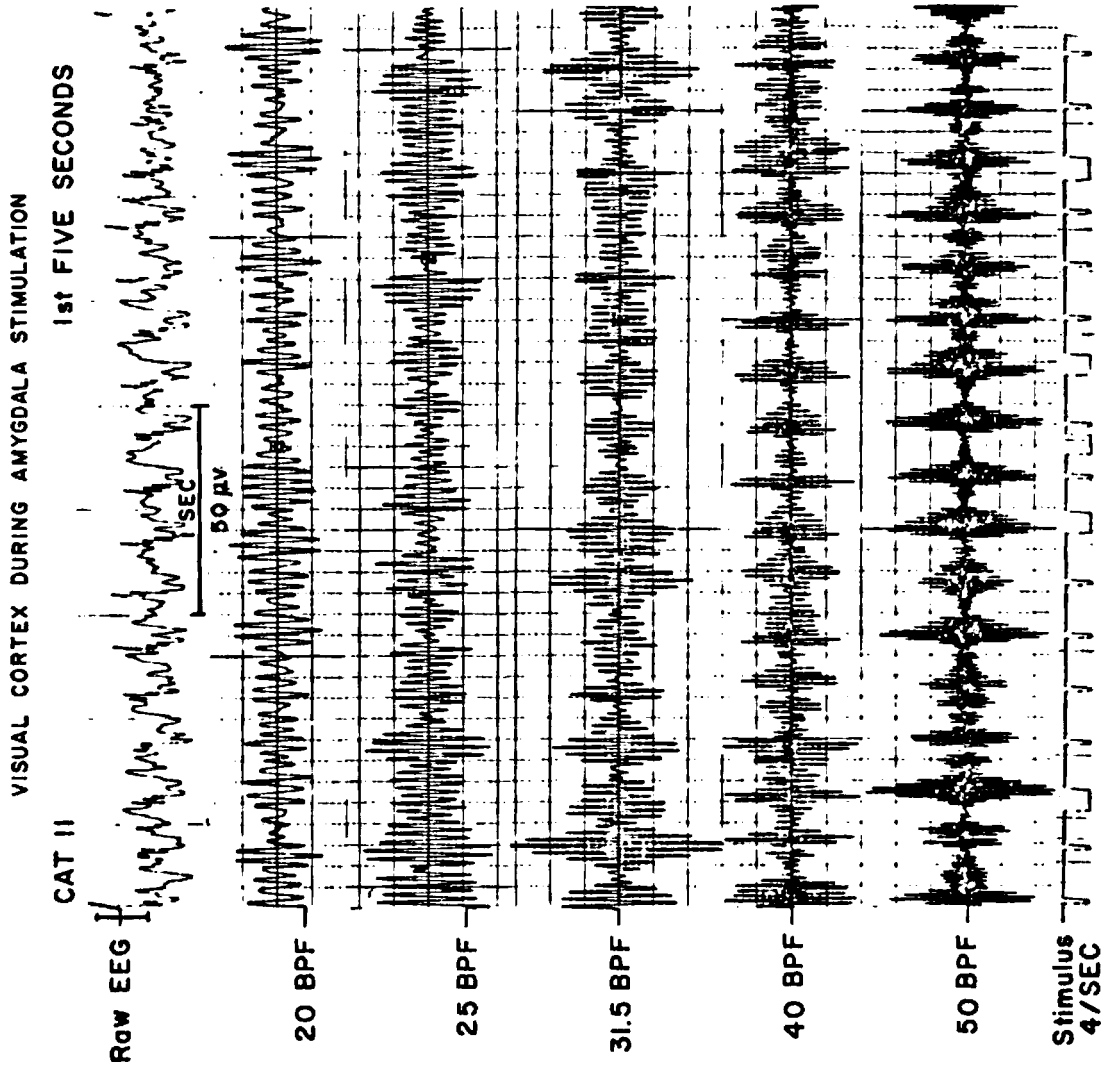

FIGURE 29

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 11 DURING THE FIRST FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

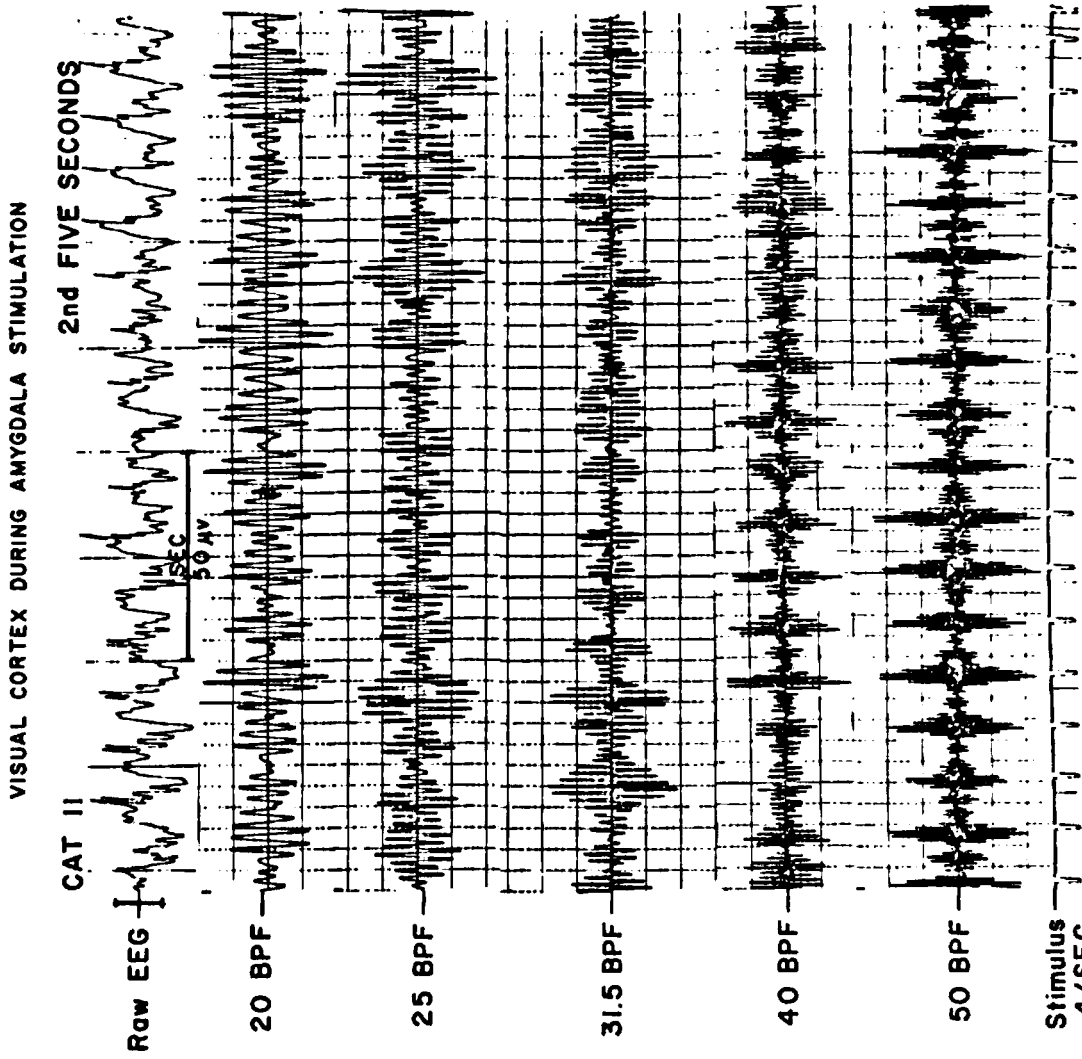

FIGURE 30

BAND-PASS FILTER OUTPUTS FOR THE VISUAL CORTEX OF CAT 11 DURING THE SECOND FIVE SECONDS OF AMYGDALA STIMULUS PERIOD

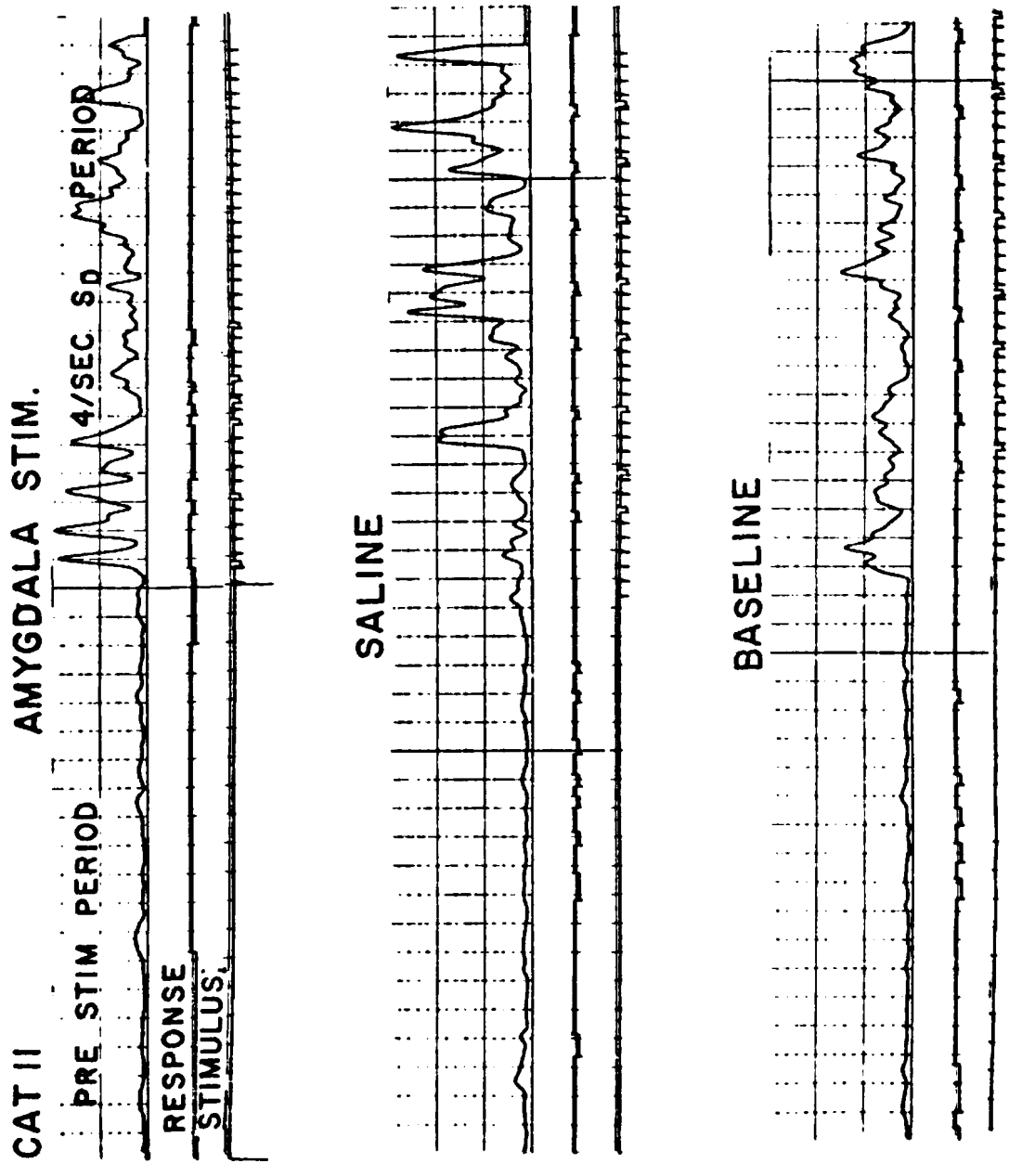

FIGURE 31

CROSSPOWER FUNCTION BETWEEN LATERAL GENICULATE AND VISUAL CORTEX OF CAT 11 DURING AMYGDALA STIMULATION, SALINE, AND BASELINE CONDITIONS

the stimulus only in the amygdala stimulation condition. Late coherence increases in the saline condition did appear, but not until almost four seconds into the stimulus period.

Experiment 2

Experiment 2 consisted of an attempted replication of the amygdala stimulation facilitation effect seen in Experiment 1. Following the completion of the schedule of experimental administrations for all brain loci in Experiment 1, Cats 9, 10, and 11 were re-injected in the amygdala. The results are found in Table 3 and represented in Figure 3. Improvement in performance was observed in all three animals at 24 and 48 hours.

CHAPTER V

DISCUSSION

This section will examine the results presented in Chapter IV within a context of the loci of stimulation, with the final segment of the chapter devoted to relating these findings to current theoretical considerations as to the structures and mechanisms underlying the presence or non-presence of facilitation effects on performance variables. The characteristic EEG patterns observed with amphetamine injection will be examined as well.

A condition, incidental to the explicit objectives of the experiment itself, was observed within one animal and is worth noting. The asymptotic pre-test ratio of Cat 9 was sufficiently high as to indicate the animal's inability to master the visual discrimination task with any degree of proficiency. Although his performance stabilized, his level still could be classified as "learning deficient". The animal's general behavior within the test chamber was hyperactive, with an inability to concentrate his attention on the stimulus. When the stimulus was present and the animal's activity brought him into proximity with the light, Cat 9 showed evidence of having made the association with the stimulus by bar-pressing. However, characteristic of

this pattern of responding was the inability of the animal to inhibit responding following cessation of the photic stimulus. When administered amphetamine, the animal displayed unusually large learning gains at 24 and 48 hours. This increase was most evident in the amygdala injections; however, posterior hypothalamic and systemic administrations improved his performance level as well. The observations on this animal appear to support research by Iasagna and Epstein (1970) in which hyperkinetic children were treated with amphetamine. Evidence of substantial improvement in learning deficiency was found, as well as improved ability to selectively attend individual components of the external environment. The amphetamine administration in Cat 9 sharply reduced random movement and produced behavioral patterns similar to those observed in remainder of the test animals, which had displayed no learning deficiency at their pre-test asymptotic level. This ability of amphetamine to act as a therapeutic sedative against hyperactivity is further supported by Bradley (1937), who observed the quieting effect of amphetamine on hyperkinetic children and on excited schizophrenic patients.

The wide variability in the response levels observed on stimulus days could possibly be attributed to two factors. The nature of response to stimulation could reflect a natural predisposition of each animal toward hyper-or hypo-activity. Another factor one must strongly consider is the high individual differences in response to a single dose

level administered to the group. The data suggest that either one or both factors may have been operating in this experiment.

Facilitation of Operant Performance

A consistent facilitation was observed when amphetamine sulfate was injected into the amygdala. If one gives credance to the mode of action for amphetamine as release and blockade of re-uptake of norepinephrine, then the amygdala might be expected to be quite high in endogenous norepinephrine. This postulation finds support in current research by Margules (1968) and Stein and Wise (in press). Stein and Wise have found that behavior-facilitating effects of amphetamine are mediated by the release of norepinephrine from terminals of the medial forebrain bundle and have further shown that the highest concentration of endogenous norepinephrine released by amphetamine is concentrated in the amygdala.

Margules observed large facilitation effects on a conditioned-avoidance task by stimulation of the amygdala with amphetamine.

Facilitation produced by stimulation of the posterior hypothalamic nuclei possibly can be related to the extremely high norepinephrine content located there. Although amphetamine does not act as strongly on the norepinephrine in the hypothalamus as it does on the amygdala-bound neurohumor (Stein and Wise, in press; Carlson et al., 1966),

some evidence of release has been observed (Glowinski et al., 1966; Axelrod, 1970). With the diffuse collaterals between the amygdala and posterior hypothalamus (Sheer, 1961), a reciprocal effect might be expected between these two areas. Facilitation from the hypothalamus might be due to indirect innervation of the amygdala by hypothalamic efferents pathways. If such a situation were tenable, then hypothalamic stimulation would not necessitate the release of large functional pools of norepinephrine in that brain loci for facilitation. However, validation of such a theory will require intensive research on these two structures and their reactions under the sympathomimetic agent.

The facilitation resulting from central injections of amphetamine sulfate are possibly attributable to the same mechanisms operating in the amygdala stimulation. Amphetamine encounter virtually no resistance at any point in the blood-brain barrier, and thereby is readily distributed in all portions of the brain via the extensive carvio-vascular system present there. Consequently the amphetamine could be expected to release large pools of norepinephrine in the amygdala, facilitating learning.

Failure of d-amphetamine sulfate to facilitate performance when injected into the ventral hypocampus can be related to the biochemical nature of the drug. As presented in Chapter II, current theory concerning the mode of action of amphetamine favors its effect upon functional pools of

available norepinephrine (Stein and Wise, 1969). If this is a tenable position, lack of facilitation resulting from hippocampal stimultion may be related to lack of effect on norepinephrine there. This hypothesis receives support from a number of studies in which amphetamine was found to have virtually no effect on hippocampal norepinephrine (Carr and Moore, 1969; Axelrod, 1970; Cahn and Herold, 1970; Fog et al., 1968; Fog, 1969).

Failure of thalamic stimulation to produce facilitation is more difficult. LeDouarec and Neveu (1970) reported that amphetamine sharply depressed thalamic action; however, depression of the centre median of the thalamus would be expected to disinhibit its inhibitory function and produce activation. Szerb (1967) found that methamphetamine injected into the thalamus produced an increase in the neurohumor, acetycholine, but did not increase EEG activity. Lack of a clear result from this brain locus would suggest a reexamination of the variables operating here-dose level, nature of the task, cannula placement, etc., and additional emperical research.

EEG Considerations

Comparison of the saline and amygdala stimulation electrical activity indicated that amphetamine tended to "sharpen" the frequency range in those band-pass filter outputs displaying large power gains coincident with the photic stimulus. By "sharpening" is meant that the largest

power gain was narrowly confined in time to coincide with the photic flash of the conditioned stimulus. The wave forms between flashes was characterized by very low power.

The frequency ranges reflecting the greatest power gains in the lateral geniculate bodies for the animals examined were 31.5 and 40 c/sec. with a pronounced shift to the 40 c/sec. activity following amphetamine stimulation in the amygdala.

The electrical activity of the visual cortices was characterized by patterns similar to those seen in the lateral geniculate bodies. Previous research (Grandstaff, 1965; Sheer et al., 1966) has indicated the presence of 40 c/sec. activity in the visual cortex during a stimulus period following acquisition of a visual discrimination task. The relationship between this frequency range and consolidation of a task criterion has also been supported in other brain loci (Bowland, 1959; Bakhiulina, 1960). It is not the intention of this author to imply that a static frequency range is associated with learning. The presence of these relationships in the 40 c/sec. frequency band characterizes a dynamic range of upper frequencies associated with EEG activation. The flexibility of this range would account for occasional prevalencies of the 50 c/sec. bands in the visual cortex during stimulus periods.

The results drawn from these analysis indicate that amphetamine sulfate has a "sharpening" effect in that frequency range characterized as related to learning, as well as narrowing

the range of the dynamic system into the proximity of 40 c/sec. activity.

These EEG patterns would appear to be related to behavioral correlates of high recognition of the stimulus as a secondary reinforcer. The crosspower functions computed bear out this contention. The frequency band displaying the highest degree of coherence between the lateral geniculate and visual cortex of each animal was the 40 c/sec. activity, and further, the amygdala stimulation produced coherence values considerably greater than did saline or the baseline condition. If one accepts the existence of a dynamic frequency range associated with learning, then certainly, amphetamine sulfate is correlated with increased learning and a consolidated frequency pattern along the primary imput pathways for the stimulus.

Obviously a distinction must be made between performance and learning. The electrophysiological data indicate a high degree of recognition of the photic stimulus as a reinforcing cue; however, operant performance on the stimulation day was frequently poor. Observations by the experimenter indicated all stimulated animals attended the stimulus with apparent diligence; however, the broad spectrum of autonomic activation accompanying amphetamine administration could be considered as a disruptive influence on an animal's ability to precisely determine onset and termination of the stimulus. Errors made under the influence of amphetamine were primarily concentrated both immediately before and after stimulus periods.

All animals appeared to be strongly alerted to the stimulus and displayed only irregular periods of virtually flawless performance within the experimental block of trials. These inconsistencies disappeared at 24 and/or 48 hours.

The inability of amphetamine to facilitate performance on the stimulation day might well be a function of an inappropriate dose level for those animals showing decrements. Certainly these findings indicate the need for additional research, in which dose levels for the selected drug are individually established for the tolerance limits associated with each subject.

In conclusion, it was felt that all major objectives established for this experiment were satisfied, and that future research might concentrate on individual components of this design to expand its findirgs.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The behavioral and electroencephalographic (EEG) effects of d-amphetamine sulfate injection into the basolateral amygdala, posterior hypothalamus, ventral hippocampus, and medial thalamic area were compared with a saline control injection, and a central administration of amphetamine delivered via the interperitoneal cavity during a behavioral task. The order of drug administration to the respective areas in each of six adult male cats was balanced in a latin-square design. The experimental task consisted of a 50 trial complex go-no-go visual discrimination task. Each trial consisted of a 10 second reinforcement period (SD) cued by a 4 c/sec. flashing light. Bar pressing responses made during the SD period were reinforced with milk. The intertrial period was a 30 second variable interval schedule during which no reinforcement could be obtained. Quantitative measures obtained on all subjects consisted of total responses, reinforced responses (SD_R), and intertrial responses (ITI_R). Daily performances were evaluated on the basis of a ratio of intertrial interval to reinforced responses (ITI_R / SD_R). In addition EEG measures were obtained from electrodes implanted in various brain loci and permanently stored on tape. EEG records were obtained on the stimulation day, while operant levels were recorded 24 and 48 hours following stimulation as well.

Comparisons were made between baseline, saline, and experimental conditions for the operant behavioral results as follows:

(1) Amphetamine stimulation in the basolateral amygdaloid nuclei was characterized by significant facilitation of performance at 24 and 48 hours following stimulation.

(2) Posterior hypothalamic stimulation resulted in significant increases in performance at 24 and 48 hours, although not of the same magnitude as the amygdala results.

(3) Hippocampal and thalamic stimulations with amphetamine were found to be non-significant in their effects on operant responses.

(4) Central stimulation with amphetamine produced operant levels which trended toward facilitation at 24 hours and reached significance at 48 hours.

(5) Saline administrations were found to be non-effective in influencing operant responding, differences between the saline control and baseline being non-significant.

Analyses of five frequency bands in the EEG arousal spectrum were performed, concentrating solely on the amygdaloid stimulation days, with examination of brain loci confined to the lateral geniculate and visual cortex, the primary projection pathways for the visual stimulus. The principle results were as follows:

(1) Amphetamine stimulation in the amygdala produced

shifts in the frequency bands resulting in a dominance of power gains coincident with the photic stimulus pulsations in the band characterized by a center frequency of 40 c/sec. This result was seen in both the visual cortex and lateral geniculate.

(2) Crosspower functions which measured the coherence of power in a frequency band for the two areas was calculated with the result that again the 40 c/sec. band was prevalent.

The results of these findings were related to the psychopharmacological properties of amphetamine and its actions on norepinephrine, as well as neuroanatomical structures in the brain possessing high concentrations of norepinephrine. The amygdala and hypothalamus were implicated as unusually active in this context. Further implications were drawn regarding the prevalence of 40 c/sec. electrical activity as an electrophysiological correlate of learning.

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APPENDIX A

ORDER OF PRESENTATION OF EXPERIMENTAL CONDITIONS

CONDITIONS

CATS	Sal.	Post. Hypo.	Amy.	Thal.	Hippo.	Cent.
2	2	6	5	1	4	3
7	5	3	2	4	6	1
8	4	1	6	2	3	5
9	3	4	1	5	2	6
10	6	5	4	3	1	2
11	1	2	3	6	5	4