THE EFFECT OF TWO BENZODIAZEPINES ON THE ELECTRICAL ACTIVITY AND DENDRITIC SPINE DENSITY OF THE CAT CEREBRAL CORTEX

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ABSTRACT

Librium® and Valium® were administered to cats in chronic high doses in order to study their effects on the spontaneous electrical activity and dendritic spine densities of the cerebral cortex. The spontaneous electrical activity monitored from a pial in-dwelling electrode assembly after Librium® administration showed no change in mean frequency. Valium® administration resulted in an increase in fast activity. Analysis of dendritic spine densities from seven cerebral cortical areas indicated that selective increases and decreases occurred. Various reasons for these changes have been suggested.

LITERATURE REVIEW

GROSS ANATOMY OF THE CAT CEREBRAL CORTEX

Anatomical Topography

The surface topography of the cerebral hemispheres of the cat has been described by Bures et al. (1967) and is shown in Fig. I of the Appendix. The nomenclature of scheme gyri and sulci of the cat brain has been generally accepted by several authors (Kappers et al., 1960; Papez, 1929; Reighard and Jennings, 1951; Taylor and Weber, 1956).

However a slight disagreement in terminology of two areas has been recorded. Bures et al. (1967) referred to the most medial gyrus on the dorsal surface of the cat's cerebral hemisphere as the lateral gyrus. Doty (1967) reviewed all the literature on nomenclature and concluded that the term marginal gyrus was correct. Similarly the term sylvian sulcus as used by Bures et al. (1967) has been called the pseudosylvian sulcus in the cat brain (Rose, 1949; Woolsey, 1960).

Functional Topography

Cerebral hemispheric areas have specialised functions which are species specific. Although some areas of the cat brain have not been completely correlated to a known function most areas have been accurately analysed.

Olfactory area. Le Gros Clark (1951) reported that selective sensitivity for odor discrimination in specific areas of the olfactory lobe and tract have related functional areas in the cortex of the prepyriform lobe.

Auditory area. Rose (1949) studied the auditory areas of the cat. He reported that the primary auditory area, which he designated as AI, was located within the midectosylvian gyrus. This functionally specific cortical area had specific afferent thalamic connections. The transitional second auditory area, AII, was located within the anterior sylvian gyrus. The posterior ectosylvian gyrus, Ep, had a similar transitional auditory function. Woolsey (1960) in his review concluded that the areas described by Rose were correct in a general sense, but more extensive study was required. The suprasylvian gyrus was reported to be an association area to both the primary auditory and the visual areas (Wilson, 1968).

Visual areas. Bilge et al. (1967) called the middle and posterior portions of the marginal gyrus visual areas I and II respectively. These areas received the afferent input fibers from the dorsal lateral genticulate nucleus. Their synapses in areas I and II were noted to have efferent fibers to other areas of the ipsi- and contralateral hemispheres. The posterior gyrus, called visual area III, received input

fibers from the medial genticulate nucleus and synapsed with neurons which had efferent fibers to area I (Wilson, 1968).

Sensorimotor area. It has long been established that the sensorimotor area of the cat brain is situated in the posterior sigmoid gyrus. Mountcastle (1957) reported that single neurons in this gyrus were functionally localised with respect to sensory input and motor output. All sensory input, except olfaction, synapsed in the thalamus before proceeding to the cerebral cortex (Ruch, 1969).

DENDRITES OF CEREBRAL CORTICAL NEURONS

Cajal (1894) as cited by Sholl (1953), in his support of the neuron theory, was one of the first to explain the structural correlation between cerebral cortical dendrites and the soma of the neuron. The Golgi Rapid Method (Golgi, 1878, as cited in Humason, 1967) for silver impregnation of neurons was modified by Cajal and others and this basic technique is still used to stain tissues for examination by light microscopy. Sholl (1953) initiated modern quantitative methods for analysing dendritic organizations in the visual and motor cortices of the cat. The main dendritic shaft which emerged from the perikaryon was termed the stem zone, the final order of branches the terminal zone and the intermediate branches the branching zone (Mungai, 1967).

Morphology

Dendrite branch density and geometry. Sholl's (1953) technique of concentric circles of increasing diameter around the soma measured length, diameter and branch density of the dendritic tree. Dendrite branch length was found to vary with the type and location of the neuron within the cortex. The terminations of observable apical dendrite trees of pyramidal cells extended 0.5mm to 1.8mm from the soma and basilar

dendritic trees and trees of stellate neurons extended between 0.25mm to 0.50mm from the cell body.

Sholl (1953) and Bok (1959) noted that cerebral cortical dendritic branching is dichotomous. Bok (1936) defined a dendritic section to be the length of a dendrite branch between successive bifurcations. Bok also studied dendritic densities and branch lengths. The results of these studies were later contradicted by the work of Sholl (1956).

Ramon-Moliner (1962) reported that stellate neurons could be classified by the morphology of their dendrites. The most common type of branch pattern was radiate with distal branches longer than proximal ones. Modified branching occurred in sensory nuclei with tufted branches, perhaps as a consequence of evolutionary change.

Dendritic geometric domains were observed to exist in a variety of shapes. Dendrites of stellate neurons were linear, which occupied a tissue space of an elongate cylinder, or planar, which assumed a diameter which was greater than its depth of field. Globus and Scheibel (1967d) observed that dendrites of pyramidal cells were always oriented in an elongate cylinder. In contrast, Mungai (1967) observed that apical dendritic fields occupied conical fields. These classification schemes were developed in an attempt to explain the

ability of certain geometric dendritic domains to accomodate afferent input.

Dendrites of pyramidal cells. Hamlyn (1963) in an electron microscope study reported that dendrites were cytoplasmic extensions of the nerve cell body. They contained granular and smooth endoplasmic reticula, mitochondria, neurofilaments and Golgi apparatus. His observation that dendritic spines were sites of synaptic contact corroborated the earlier work of Gray (1959a).

Mungai (1967) reported that the greatest density of dendritic spines was found in the terminal zone, with smaller densities in the stem and branching zones. The dendritic spine of the pyramidal cell consisted of a stalk and a large terminal ovoid bulb (Jacobson, 1967).

Other ultrastructural studies have shown that the post-synaptic membrane of the dendritic spine can exist in one of two structural forms (Gray, 1963; Colonnier, 1968). Type I synapses had an increased electron dense area over most of the presynaptic cleft while Type II synapses had a thickened region over a small portion of the cleft. Bodian (1966) theorized that differentiation of synaptic function was possible as a result of structural differences of synaptic vesicles.

Dendrites of stellate cells. Ramon-Moliner (1961) reported that stellate cells had extremely thin dendrites with few spines. Their dendritic trees formed flattened disks or elongate cylinders in the tangential plane. Studies by Colonnier (1964) and Mungai (1967) corroborated these observations. Wong (1967) found approximately equal numbers of circular and oval dendritic fields in cat auditory cortex. He theorized that the variation was correlated to function, not to mechanical distortion.

Colonnier (1967) observed bulbous enlargements randomly placed along the dendritic branches of stellate neurons. Mungai (1967) reported that these bulbous enlargements occupied 47 per cent of the total surface area of the dendritic tree of a stellate neuron.

Functional Significance

Most synaptic contacts to the neuron have been found to occur on the dendrite (Sholl, 1953; Marin-Padilla, 1967; Globus and Scheibel, 1967d). As dendritic branches intermingle with many other neuronal fibers, many functional contacts were possible (Cragg, 1967). These could be axodendritic, axo-spinodendritic (Gray, 1959b), or dendrodendritic (Famiglietti, 1970).

The complexity of dendritic branching may be correlated to dendrite function. Neurons whose dendritic trees occupied a small horizontal diameter may have a localized function whereas neurons whose dendrites formed a wide plexus were associative, with several vertical neuronal processes, in function (Eliseyeva and Durinan, 1968). Similarly, Colonnier (1964; 1967) emphasized the functional importance of a vertical columnar organisation of neurons and neuronal processes in the cerebral cortex.

Structural differences of synaptic sites may indicate functional variations. Gray (1959b) suggested that some axodendritic synapses were excitatory while others were inhibitory; axospinodendritic synapses were excitatory. Dendrodendritic synapses were likely inhibitory (Famiglietti, 1970). Marin-Padilla (1968) found that synapses en passant, in which an axon closely parallelled a dendrite section and synapsed with more than one spine, might be of great functional significance in the cerebral cortex.

This would seem to indicate that the dendrite was involved in the assimilation of the afferent input to the neuron. One reliable method of studying the function of nervous tissue has been to experimentally alter or damage some of the selective cells and to compare the resulting changes with that seen in control tissues.

Environmental manipulation of afferent input. Environmental alterations resulted in changes of the fine structure of the dendrite. Diamond et al. (1964) found that an enriched environment resulted in an increased cortical depth in rats, presumably due to increases in dendrite branching, in size of neuronal soma and vascularization. The controls in Diamond's study had been environmentally isolated. Observations by Holloway (1966), also using isolates as controls, corroborated the earlier findings of Diamond.

Valverde (1967) observed that newborn mice reared in darkness had a significant reduction in the number of spines which developed in dendrites of the visual cortex. Coleman and Riesen (1968) studied the effects of light deprivation in newborn kittens. They reported a significantly smaller number of developing dendrite branch points with shorter dendrite branch lengths when compared to controls. Shapiro and Vukovich (1970) reported that an increase in environmental input by gentle handling of neonate rats resulted in increased dendritic branching and spine density by the eighth day.

Surgical deafferentation. Hedley Jones and Thomas (1962) found a significant decrease in dendrite density in the cerebral cortex as a consequence of transection of the olfactory bulb in rats. Globus and Scheibel (1967b) studied the effect

of enucleation of visual tracts in newborn rabbits. A reduction in spine density was noted to occur along the branching zone of the apical dendrite in the olfactory cortex. They later (1967c) hypothesised that the loss of spines was the result of a loss of afferent input from synaptic terminals which impinged upon them. They observed also (1967a) that some spines had an abnormal termination which could be a variation of either length, straightness or spine direction.

Valverde and Esteban (1968) studied the effects of enucleation of mouse cortical tissue on spine density. Functionally adjacent cortical tissues to the enucleated area were noted to have a reduced spine density while the contralateral hemispheric area, connected by transcallosal fibers, remained unaltered.

Chronic neuronal isolation in adult cat brains (Weisman et al., 1967; Weisman and Pinsky, 1970; Weisman, 1970) resulted in significant decreases in dendrite branch density and spine density. They reported also that cortical dendrites exhibited limited morphological plasticity. A significant increase in spine density was reported to occur as a result of direct electrical stimulation which far exceeded normal neurophysiological drive.

Physiological basis - the dendritic potential. It is well known that dendrites exhibit a constant, characteristic change in electrical potential following electrical direct surface stimulation. Chang (1951) showed that the initial response to a stimulus input originated from the apical dendrites. The recording of the response consisted of a positive deflection followed by a prolonged negative wave. The maximum potential change was recordable adjacent the stimulus point with decremental potentials recorded at successively distant points (Chang, 1951; Frank and Fuortes, 1961).

Dendritic potentials spread electrotonically with an extremely slow rate of conduction due to efflux and influx of chloride ions. The dendritic potential has a duration rate of 15 - 20 msec., no absolute refractory period, and is capable of summation. This is in contrast to the all-or-none response characteristic of axons (Rall, 1957; Clare and Bishop, 1955).

It has been suggested that dendritic potentials are the most primitive type of response (Clare and Bishop, 1955). Jacobson and Pollen (1968) have found that these potentials selectively regulated neuronal impulse transfer. This regulation was due to the decremental nature of the impulse transfer whereby only two to three per cent of the magnitude of the potential at distal dendrite branches reached the soma.

Dendritic potentials reached the soma of the neuron following summation with successive axonal inputs and capacitor discharges at dendritic branch points (Frank and Fuortes, 1961).

Electroencephalography has demonstrated that dendritic potentials are related to the spontaneous activity of the cortex (Grossman, 1955; Deza and Eidelberg, 1967). The spontaneous activity was correlated to the ontogenetic development of complex dendritic trees (Flexner et al., 1950; Grossman 1955; Eayrs and Goodhead, 1959). The spontaneous activity is significantly reduced temporarily after electrical stimulation (Bishop and Clare, 1952) and after surgical cortical isolation (Kristiansen and Courtois, 1949; Burns, 1950; 1951; Frank and Pinsky, 1964).

ELECTROENCEPHALOGRAPHY

Electroencephalography is a method of recording the spontaneous electrical activity of the cerebral cortex. Berger (1929) reported that electrodes in contact with the human scalp monitored the fluctuations of the electrical potential and could therefore be used as an indicator of general health. Cooper et al. (1969) critically reviewed techniques which were developed to record and to analyse patterns of brain electrical activity. Electrical leads may be monopolar, where the active lead was in reference to an indifferent electrode, bipolar or multipolar where the active lead was in reference to another potentially active electrode. Multipolar and bipolar leads are most commonly used. Bipolar leads indicate cortical electrical activity of two points in reference to each other.

Gibbs et al. (1940) and Engel et al. (1944) developed quantitative methods of analysing the electroencephalogram (EEG) since gross observations were not sufficiently accurate to determine significant variations. Gibbs measured changes in absolute energy of the wave form. Engel's method of measuring the distribution of different wave frequencies from a continuous EEG recording could be used to determine the most

common frequency or the frequency of individual wavelengths in a given segment. The latter method was more accurate but more tedious.

Marshall (1955) developed a simple ruler with which the length of each wave could be accurately measured. This ruler is now considered as the standard means of manual analysis of EEG frequency spectra (Saunders, personal communication).

Patterns of human electrical brain activity were described by Glaser (1963). At rest alpha (α) rhythm predominates, with frequencies of 8 to 13 cycles per second and amplitudes of 25 - 100 mvt. Beta (β) activity, with frequencies of 13 - 20 cycles per second appears in response to mental activity. Theta (θ) waves, 4 - 7 cycles per second are not common. Delta (δ) waves 0.5 - 3.5 cycles per second are associated with light sleep. Other wave forms seen may be desynchrony of the above patterns. Fast activity, with waves of high frequency and low amplitude, termed low voltage fast (LVF), are seen during periods of activated sleep (Dement, 1958) and during barbiturate sedation (Chafetz and Cadhilhac, 1954).

Recording electrical activity directly from the cortex involved placing leads on or within the cortex of animals. The electrocorticogram (ECoG) in cats produced comparable

results to the EEG but with higher amplitudes (Kido et al., 1966). Behavioral excitation resulted in an increased frequency (Kido et al., 1966). The frequencies changed from predominantly beta at rest to predominantly alpha during light sleep to delta waves during deep sleep and to fast activity during activated sleep. Chemical stimulants increased the frequency and amplitude of the electrical pattern (Chafetz and Cadhilhac, 1954; Vastola, 1961).

Electrical activity of the cerebral cortex must be the result of a complex and intricate mechanism. The physiological basis is not yet clearly understood. Dubner and Gerard (1939) theorized, on the basis of gross observations, that the EEG was due to a synchronous discharge of unspecified cortical cells. Ruch et al., (1969) stated that the EEG resulted from a reverberating synchrony within a closed circuit.

BENZODIAZEPINES

Introduction

Drugs have been defined as chemical agents that affect protoplasm (Fingl and Woodbury, 1970). The effectiveness of any drug is dependent initially upon factors which affect its rate of absorption in the animal. Schou (1961) stated that this was related to the rate of blood flow, its passage across cell membranes and the properties of the drug vehicle. Schanker (1962) reviewed further the studies of absorption and stated that the passage of the drug through a membrane was selectively effected by either a diffusion or an active transport mechanism.

Bowman et al. (1968) reviewed the results of several studies on drug distribution. He stated that blood is the major transport medium. Sequestration was believed due to the nature of the transport vehicle, differential pH at specific sites and the availability of drug receptors.

The active state of a drug varies in many respects. Fingl and Woodbury (1970), in discussing the effectiveness of any drug, concluded that some drugs were active in their original state while others became active as a result of biotransformation. Similarly they stated that biotransformation may convert an active drug to a metabolite which is also

active. The excretion of drugs therefore involved the removal of the drug in its original and altered states.

Drugs which act on the cerebral cortex must pass through the blood-brain barrier. The most effective drugs which do this are lipid-soluble (Sherman, 1970).

Pharmacology

In 1961 Sternbach and Reeder reported that they had synthesized 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide, the first of a series of organic compounds which have been classified as major antianxiolytic agents (Fig. II of the Appendix).

Randall et al. (1960; 1961) initiated a comprehensive pharmacological study of the benzodiazepine derivatives. The parent compound, methaminodiazepoxide, also known as chlordiazepoxide, was found to have the following properties: muscle relaxant (Randall, 1960; T. Harris 1960) effective anticonvuisant (Randall, 1960; T. Harris 1960) Schallek et al., 1964; Sawyer et al., 1968); effective taming agent (Randall, 1960; Randall et al., 1960; Randall and Kappell, 1961; Heise and Boffe, 1961); appetite stimulant (Randall, 1960; Randall et al., 1960; Ayd, 1962); anxiolytic (T. Harris, 1960; Randall et al., 1960; Randall and Kappell, 1961; Ayd, 1962; Winfield, 1963).

Diazepam, the first congener of chlordiazepoxide which was synthesized, was five to ten times more potent in respect

to its anticonvulsant activity (Randall et al., 1961) and four times more effective as a muscle relaxant and calming agent as chlordiazepoxide. Neither drug appeared toxic in dogs after a six-month study.

Oxazepam, a metabolite of diazepam as well as a synthetic congener of chlordiazepoxide, was similar to diazepam in effectiveness (Gluckman, 1965) but only at a dose level higher than that required for chlordiazepoxide (Tobin et al., 1964).

Benzodiazepines have a broad spectrum of activity yet their mode of action is not yet fully understood. Some investigators (Winfield and Aivazian, 1961; Arrigo et al., 1965; Hernandez-Peon and Rojas Ramirez, 1966) have suggested that the drug acted directly on neuronal cortical cells since the drug modified spontaneous electrical activity.

Schallek and Zabransky (1966) stated that the drug effect was at least partly due to electrical depression of the hypothalamus. Killam (1962) found that the reticular activating system would be blocked only after high doses of chlordiazepoxide. Ngai et al., (1966) stated small doses of benzodiazepines were effective in blocking spinal reflexes by acting upon the reticular formation. Schallek et al. (1962; 1965) found that sleep was induced in cats at a dose level

of 10mg. per kg. b. wt. i.m. of chlordiazepoxide. This dose level gave typical large slow waves from the cortex which indicated that the limbic system was affected. Svenson and Gordon (1965) reported that low doses, 1 - 2 mg. per kg. b. wt., of diazepam did not depress cortical activity but at 5 mg. per kg. b. wt. i.m. the cerebral cortex, hippocampus, amygdala and septum were all electrically depressed to produce ataxia and sleep.

Metabolites of benzodiazepines are known to exhibit animal species specificity. Koechlin et al. (1965) found that chlordiazepoxide was biotransformed to oxazepam in dogs and man. Rats did not form oxazepam as a metabolite (Schwartz, et al., 1963; 1965; Schwartz and Postma, 1968; Marcucci et al., 1970). In contrast, Kvetina et al. (1968) found oxazepam as a metabolite of diazepam in rats.

In all animal species that have been studied, benzodiazepine uptake is fast, with only traces remaining in the body after 48 hours (Schwartz et al., 1965; Placidi and Cassano, 1968). Drug biotransformation occurred in the liver (Schwartz and Postma, 1968).

Jori et al. (1969) found that benzodiazepine derivatives potentiated the action of phenobarbital. The major clinical side effects observed in adults were drowsiness and ataxia

(Hare, 1963). Svenson and Gordon (1965) found that fast activity of the EEG persisted after the drugs were discontinued. Gluckman (1965) and Ryan et al. (1968) found that these drugs may increase behavioral depression. Fox et al. (1970) and Salzman et al. (1969) reported that chronic low doses in mice and man increased agression.

Chronic administration of chlordiazepoxide or diazepam resulted in an increased tolerance to the drug (Taylor et al., 1969) but in general toxicity has been reported to be low (Zbinden et al., 1961; Tobin and Lewis, 1960; Smith et al., 1964). The only serious side effect from clinical cases that is reported in the literature is agranulocytosis, after administration for one week at unreported doses, but this can be determined before damage is serious (Kaeblin and Conrad, 1960).

Electrical Studies

Several different studies on the cortical electrical activity following benzodiazepine administration has yielded incongruous results. Randall et al. (1961) stated that chlordiazepoxide and diazepam at a dose level which caused sedation would also depress the EEG frequency in rats. Sedation to a sleep level was induced by diazepam at 5 mg. per kg. b. wt. and chlordiazepoxide at 10 mg. per kg. b. wt. Randall and Schallek (1967) observed that in the cat neither

drug affected the recorded electrical activity from the ectosylvian and suprasylvian gyri at a dose of 10 mg. per kg. b. wt. i.v.

Requin et al. (1963) in acute ECoG studies of cat cerebral cortex found that chlordiazepoxide at 1.5 mg. per kg. b. wt. i.v. resulted in fast activity while diazepam at 1 - 2 mg. per kg. b. wt. i.v. increased the amplitude of fast activity. Kido et al. (1966) stated that ECoG recordings in cats with acutely implanted electrodes differed significantly from an EEG; ECoG recordings from chronically implanted electrodes were similar to the EEG in frequency but were of greater amplitude. They found that chlodiazepoxide induced characteristic behavioral light sleep at 20 mg. per kg. b. wt. p.o.

Schallek and Kuehn (1965) found that chlordiazepoxide at 10 mg. per kg. p.o. in cats with chronically implanted electrodes produced ataxia and significantly increased the frequency of the ECoG from 8.5 to 10.3 cycles per second. Diazepam significantly increased the ECoG frequency from 8.7 to 12.1 cycles per second after 5 mg. per kg. b. wt. p.o.

STATEMENT OF THE PROBLEM

Alteration of the afferent input to the cerebral cortex by manipulation of environmental conditions has resulted in changes in dendritic structures. Sensory deprivation of neonates results in selective decreases in dendritic branches and in spine densities. Environmental enrichment results in an increased maturation rate of cerebral cortical dendrites in neonates. Chronic surgical deafferentation results in significant decreases in the density of cortical dendrites. These decreases were attributed to the reduction of spontaneous electrical activity within the cortical isolate.

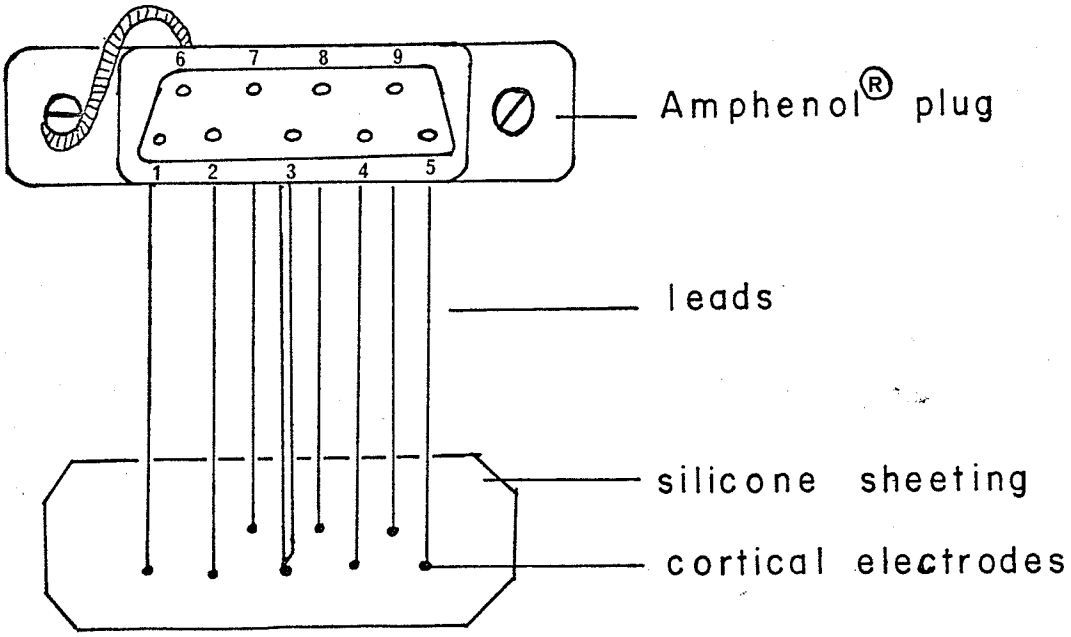
This research project has had a two-fold purpose: to study possible changes in the spontaneous electrical activity and in dendritic spine densities in cat cerebral cortex after chronic administration of high doses of two benzodiazepines, chlordiazepoxide (Librium^{R}) and diazepam (Valium^{R}). Dendritic spine densities of pyramidal neurons from seven functional cortical areas would be calculated after histological impregnation with silver.

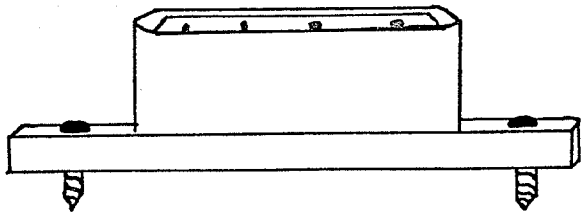
MATERIALS AND METHODS

Twenty-two adult cats (1.3 - 3.4 kg.) of either sex were used in this study. They were kept in the Animal Holding Facilities (A.H.F.) of the Zoology Department for two to five weeks to acclimate them to individual cage conditions and to ensure that they were disease-free. Throughout the experimental period the cats were fed commercial cat food and were exercised daily and under control conditions exhibited normal feline behavior.

ELECTRODE INSERTION ON THE PIA MATER

Each cat was familiarised with the conditions of the research laboratory then prepared for surgery. An electrical cortical recording apparatus was prepared for insertion on the pial surface of the left cerebral hemisphere. Platinum-iridium multiple electrodes were glued into silicone sheeting (Silastic^{R}) and insulated copper wires were soldered to the electrodes. The wires joined a nine-lead Amphenol^{R} plug (223-1209) which was to be fastened to the right parietal bone. Fig. 1 illustrates the structure of the plug.





Side view of Amphenol® plug

Figure 1. Structure of the in-dwelling electrode apparatus and plug. The exterior measurements of the Amphenol® plug are 7 x 27 mm. Lead 3 was the active reference electrode for all recordings.

Surgical technique

Cats were anaesthetised with an i.p. injection of sodium pentobarbital (Nembutal^{R}) at a standard dose level of 35 mg. per kg. body weight. When stage III anaesthesia was induced the animal was transferred to an operating table, placed in a supine position, restrained by rope stays and the head immobilised in a Czermak^{R} holder.

Hair was shaved from the head and neck regions and the skin disinfected with 70% ethyl alcohol.

The tissues were kept moist at all times with warm physiological saline. A dorsal midline incision extending from a point anterior to the parietal suture to a point beyond the lambdoidal ridge was made with a #22 scalpel blade. The median raphae was reflected with the left temporalis muscle. A curved periosteal raspatory was used to separate this muscle from the cranial periosteum; the latter was usually destroyed by the raspatory.

The craniectomy of a portion of the parietal bone measuring 20mm. X 10mm. over the midsuprasylvian gyrus was effected with a #8 vanadium tipped burr and bone rongeurs. Bone wax was used to arrest hemmorrhage from the diploe of the calvarium.

The temporalis muscle of the right hemisphere was

partially reflected to enable the Amphenol® plug to be fastened with stainless steel screws and acrylic cement to the right parietal bone.

Iris scissors and forceps and a scalpel with a #12 blade were used to incise the dura mater over the midsupra-sylvian gyrus. Silicone sheeting with the electrodes was inserted on the pia mater and in some instances the cut edges of the dura mater were sutured together with 000 nylon thread. Gelfoam® was placed around the wire leads and over the exposed dura mater. This was covered with Gelfilm®. Acrylic cement was placed over the Gelfilm and cut edges of the bone. A strip of Gelfilm was placed over the Acrylic cement, the temporalis muscle returned to its original position and sutured to its contralateral homologue. A topical antibiotic (Mycifradin®) was applied to the surgical area and the skin sutured together with #80 cotton thread. Penicillin G at a dose of 500,000 I.U. was given i.m. and the cat placed in a post-operative cage.

Recovery

The cats regained consciousness in six to forty-eight hours. The surgical area was cleaned regularly and treated with topical antibiotics. Approximately seven days after surgery each cat was transferred to A.H.F.

ECoG RECORDINGS AND DRUG ADMINISTRATION

ECoG recordings were obtained using a Grass® P15 preamplifier. A D52 Telequipment® D. C. oscilloscope was used for visual observation of electrical tracings and paper recordings were made with either a two- channel Brush® or Dynograph 504 B® recorder.

The cats were placed in a small animal cage 30"x8"x8" during the test period. This small cage was placed inside a copper shield area which kept electrical interference to a minimum. During the short period of behavioral adjustment to the new condition of confinement all electrical leads were tested individually and the recordings checked to find the optimum lead for each cat. Prior to each recording session the recording equipment was allowed to equilibrate and then the needle deflection of the paper recorder was calibrated to an amplitude of 5mm. equalling 50 mVt. Predrug electrical patterns of controls were made of all cats with the animals behaviorally at rest.

Three cats were used as histological controls. These were given i.m. injections of a drug-free vehicle twice daily; two cats were given 0.1 cc. of physiological saline and one cat 0.1 cc. benzyl alcohol. During the course of this study it was not possible to obtain ECoG tracings from these cats.

After twenty-one days of chronic administration these cats were killed with a 80 mg. per kg. body weight overdose of sodium pentobarbital (Nembutal®). All other cats were killed at different specified times by the same method.

Four cats were each given single Librium® injections every 48 hours. The dose level was increased by 2 mg. per kg. body weight until a dose level of 16 mg. per kg. was reached. At this dose level the cats exhibited pronounced ataxia and generally assumed a light sleep posture (Kido et al., 1966). This level of sedation was maintained with i.m. injections every twelve hours for a three-week period. ECoG tracings were recorded daily. The three cats were then killed with an overdose of Nembutal®. Injection of the fourth cat was discontinued on the twenty-first day, its behavior observed, then transferred to the A.H.F. Seventy-two days later it was killed.

The procedures used to determine the daily dose level for the Librium®-treated cats were also used on four Valium®-treated cats. A dose level of 5 mg. per kg. body weight i.m. sedated the cats to a light sleep posture with ataxia which was comparable to the Librium®-treated cats. This dose level was maintained for three weeks. On the eighth day of chronic sedation it was necessary to change the injection route from

intramuscular to intraperitoneal. After twenty-one days three cats were killed. The chronic administration was discontinued to the fourth cat on the twentieth day, the cat was observed for any behavioral changes, then transferred to A.H.F. The cat was killed 45 days later.

HISTOLOGICAL PROCEDURE

Immediately after the death of the animal cortical tissue blocks were removed by the following method. A dorsal midline incision of the scalp was made with a #21 scalpel blade. The skin, separated from the temporalis muscle, was reflected. The temporalis muscles and the Acrylic cement were removed. The entire calvarium was clipped away with rongeurs and when the cerebral hemispheres were exposed the dura mater and leads were removed. The tissues were kept moist with warm physiological saline at all times.

The location of the physical depression of the cortex from the electrodes and silicone sheeting was noted (Fig. 9). Blocks of cortical tissue were removed from specific gyri using a #11 scalpel blade (Fig. 2). Excised tissues were immediately placed in individual prepared bottles of fixative. The histological procedure used was the modified Golgi technique as described by Weisman (1970). It is outlined in Fig. III of the Appendix. A Sartorius® horizontal clinical microtome, model 27, with an A or B blade was used to cut sections 40 microns thick.

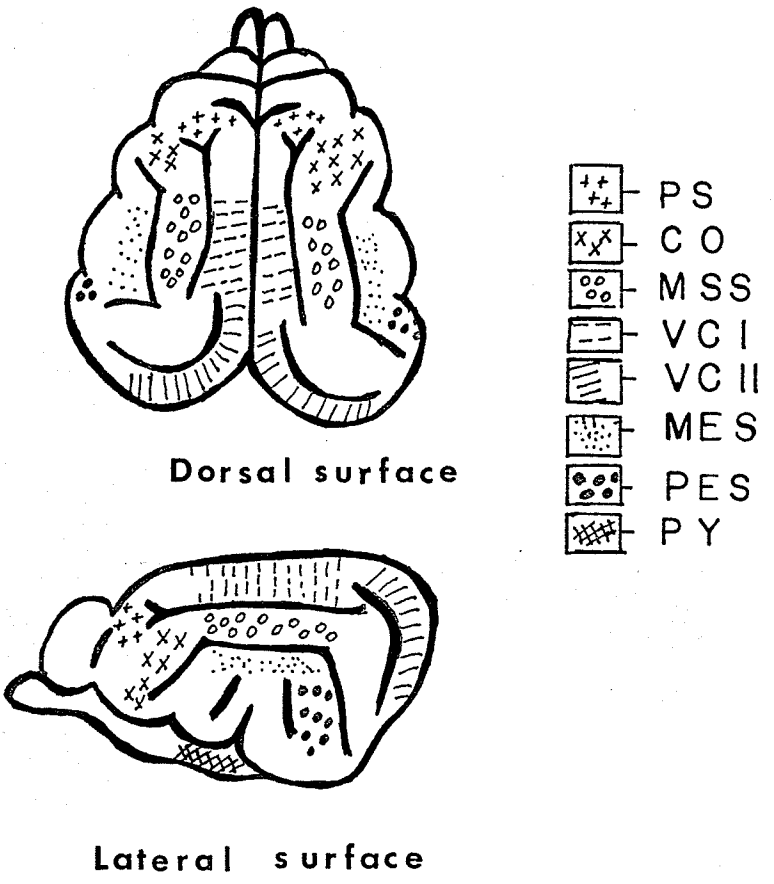


Figure 2. Dorsal and lateral views of the cat cerebral hemispheres. The areas from which tissues were excised for histological analysis are indicated by shading; Post-sigmoid gyrus (PS); Coronal (CO); Midsuprasylvian (MSS); Marginal (VC I); Posterior (VC II); Midectosylvian (MES); Posteriorectosylvian (PES); and Pyriform (PY) gyri.

ANALYSES

ECoG Tracings

The relative frequency of each cat's ECoG pattern was determined with an EEG ruler (Marshall, 1955). The technique involved measuring the distance from one peak to the successive peak of the recording (Fig. 3) and calculating the mean cycles per second from the frequency spectra.

Segments of the ECoG which were selected for analysis were free from artifacts such as muscle spikes, auditory and visual responses and corresponded to periods when the cat was behaviorally at rest. Several second intervals of the recording were analysed. The section to be measured was marked, and the distribution of the individual wavelengths was noted. The mean frequencies, and standard errors were calculated with an Ollivetti Programma 101.

Needle deflections less than one mm. were not measurable. Noise, or external interference was negligable as recordings taken from the open system indicated regular sinusoidal waves of 60 cycles per second. These were not present in the recordings. Also, as another check, recordings were made from leads in certain cats which were not functional, yet provided a ground for the system. No 60 cycle per second waves were seen.

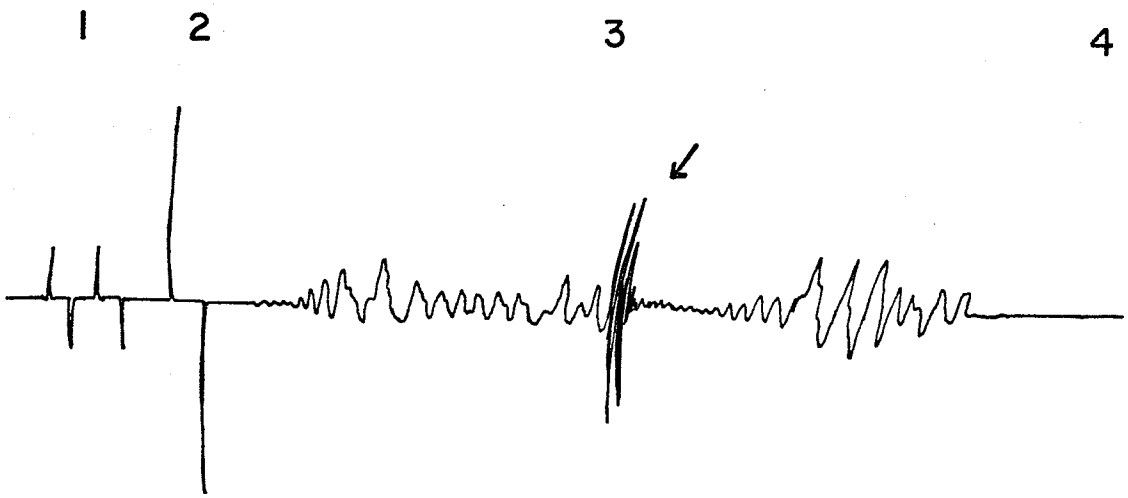
Amplitudes of the wave forms were measured by recording the height in mm. The measurements were taken in artifact-free segments for several one second intervals. Prior to all recordings the paper recorder was calibrated to a needle deflection of 5 mm. for 50 mVt.

Histological sections

Dendritic spine densities, measured as the number of spines per micron length of dendrite section, were calculated for each block of tissue. Observations were made under oil immersion X1000 with a Nikon light microscope fitted with an ocular micrometer. The most complete apical dendritic trees of pyramidal neurons were located in each section, the location and size of the soma noted, the length of the branch section and number of observable spines recorded. Stem, branch and terminal dendritic zones were analysed. Manipulation of the fine adjustment was necessary to count the spines in all focal planes.

The density of spines on basilar dendrites of pyramidal neurons was similarly determined. The stem and branch zones were grouped.

The means and standard errors were calculated and Cochran's modified t-test used to determine significant changes in spine densities under control and drug conditions.



1· Calibration 5mm= 50 uVt.
2· Manually set limit for maximum needle deflection.
3· ECo G recording.
4· Baseline check.
Arrow indicates artifact from muscle contraction. This segment would not be analysed.

Figure 3. Sample ECoG tracing for analysis. Baseline is noted before and after each recording. Amplitude is calibrated to 5mm. for 50 mVt. Maximum needle deflection limited to 20 mm. Frequencies determined for each wave within a given segment by measuring the distance between successive peaks of the waveform.

RESULTS

GROSS OPERATIVE OBSERVATIONS

Eleven of the twenty-two cats available survived the surgical procedures. Three cats showed no visible reaction to a normal anaesthetic dose of Nembutal® only on the first administration. However, most cats exhibited typical reactions of anaesthesia expected from sodium pentobarbital. Soon after injection they underwent a period of physical excitation, loss of peripheral muscle coordination which was soon followed by stage III anaesthesia. There was a loss of pupillary reflex and a negative response to the Babinsky test. Cats which showed extreme excitation which approximated a rage response following the anaesthetic injection were not used.

One week after surgery an area immediately around the Amphenol plug showed signs of mild purulent infection. Post-mortem examination showed that the infection was localised. Four of the cats had muscular convulsions within three hours of receiving i.m. injections of 500,000 IU Penicillin G. One of these cats died. The three cats were given Lincomycin® with no recurrence of convulsions. Within three weeks after surgery all cats displayed normal feline behavior with no signs of locomotor impairment. All cats responded to gentle handling by purring.

Table 1. Mean frequencies (cycles per second ± SE) of ECoG tracings of cats under control conditions.

Cat Number	Control Condition		
	No injection	i.m. injection	
		0.1 cc. saline	0.1 cc. benzyl alc.
14	24.7 ± 1.2	--	
17	22.9 ± 1.5	22.3 ± 1.1	
20	18.8 ± 1.3	19.7 ± 1.6	
21	25.2 ± 0.9	--	
13	24.2 ± 1.0	26.7 ± 1.1	
22	25.1 ± 0.8	24.1 ± 1.0	
7	22.3 ± 1.3		21.9 ± 1.0
19	24.4 ± 1.1		25.6 ± 1.2

ECoG RECORDINGS AND CHRONIC DRUG ADMINISTRATION

The preliminary check of all leads of all cats indicated that while most leads were functional, the recordings from lead #3 (see Fig. 1) were the most easily analysed in respect to amplitude and frequency. Due to technical limitations of the paper recorder the amplitude of the wave forms recorded on the paper were not accurate. Only frequencies were analysed.

The mean frequencies of the ECoG patterns obtained under control conditions from eight cats are given in table 1. The frequencies in cycles per second ranged from:

18.8 ± 1.3 to 25.2 ± 0.9 with no injection;

19.7 ± 1.6 to 26.7 ± 1.1 with 0.1 cc. saline i.m.;

21.9 ± 1.0 to 25.6 ± 1.2 with 0.1 cc. benzyl alc. i.m.

No significant differences in the frequencies were found to exist among the three groups. These results are in agreement with the values reported earlier for ECoG recordings from surface electrodes on the anterior sigmoid gyrus of cats behaviorally awake and at rest (Yamamoto, 1959; Kido et al., 1966). In this study it was not possible during the experimental period to administer both benzyl alcohol i.m. and saline i.m. to the same cats.

Chronic Administration of Librium®

Mean frequencies of ECoG patterns obtained from increasing levels of Librium® are shown in table 2. At ten mg. per kg. b. wt. all four cats appeared behaviorally drowsy. The ECoG did not show a corresponding increase in alpha and beta wave forms.

At the dose level of 16 mg. per kg. b. wt. no significant changes were found to occur in wave form of relative frequency when compared to control tracings. At this dose level the cats maintained a light sleep posture. They exhibited pronounced ataxia of the limbs but not the final characteristic ataxia with head droop. The cats had an increased appetite and could be easily aroused but not excited. They did not purr. A chronic administration dose of 16 mg. per kg. b. wt. was selected on the basis of the level of ataxia and behavioral sedation.

The mean frequencies of the ECoG patterns at a constant dose level of 16 mg. per kg. b. wt. Librium® i.m. are shown in table 3. The frequencies in cycles per second ranged from:

16.5 ± 1.2 to 23.1 ± 1.0 in cat #17;

13.6 ± 1.1 to 23.3 ± 1.0 and low voltage fast, LVF, (Dement, 1958), in cat #20;

14.3 ± 1.0 to 24.0 ± 1.1 and LVF in cat #21.

Table 2. Mean frequencies, cycles per second ± SE, of ECoG tracings at various hours after increasing doses of Librium® (chlordiazepoxide-HCl) administered i.m.

Dose level, mg per kg. b. wt.	Post-inj. time hours	Animal number			
		#17	#20	#21	Grouped
	1	-*	-*	-*	-
1	3	-*	-*	-*	-
	24	22.3 ± 1.8	18.4 ± 1.2	-*	-
2	1	24.3 ± 1.4	17.1 ± 1.1	-*	21.2 ± 1.1
	4	26.5 ± 1.3	18.9 ± 1.2	-*	23.1 ± 0.9
4	1	25.1 ± 1.2	20.6 ± 1.1	-*	23.1 ± 0.9
	4	23.9 ± 1.4	19.0 ± 1.0	-*	22.2 ± 1.1
6	1	22.9 ± 1.2	21.7 ± 1.4	-*	22.3 ± 0.9
	4	23.6 ± 1.3	19.1 ± 1.1	24.1 ± 1.1	22.5 ± 0.7
8	1	24.3 ± 0.8	18.0 ± 1.8	21.6 ± 1.1	21.6 ± 0.7
	4	25.7 ± 1.3	14.8 ± 1.2	22.8 ± 1.1	22.1 ± 0.8
10	1	23.1 ± 1.0	17.5 ± 0.8	25.6 ± 0.7	22.7 ± 0.6
	4	24.9 ± 1.0	18.5 ± 0.8	24.8 ± 1.2	23.2 ± 0.7
12	1	20.9 ± 1.4	21.4 ± 1.2	22.5 ± 1.2	21.6 ± 0.7
	4	20.8 ± 1.2	18.2 ± 1.3	19.1 ± 0.9	19.4 ± 0.7
14	1	24.1 ± 1.5	20.8 ± 0.9	23.3 ± 1.4	23.1 ± 0.7
	4	24.2 ± 1.3	25.0 ± 1.2	25.2 ± 1.3	24.1 ± 0.7
16	1	22.9 ± 1.4	17.3 ± 0.9	25.5 ± 1.4	22.8 ± 0.9
	4	21.7 ± 1.4	18.1 ± 1.4	24.9 ± 1.1	21.9 ± 0.8

* Paper recorder speed too slow for manual analysis.

Table 3. Mean frequencies, cycles per second ± SE, of ECoG tracings of animals at a constant dose level of 16 mg. per kg. b. wt. Librium® i.m. Readings are four hours post-injection except those designated " ‡ " which are after no injection.

Day	Animal number			
	17	20	21	Grouped
1	19.9 ± 1.0	15.9 ± 1.4	22.6 ± 0.9	20.0 ± 0.7
2	21.6 ± 1.0	15.7 ± 0.7	24.0 ± 1.1	20.9 ± 0.7
3	21.0 ± 1.3	16.2 ± 1.2	23.4 ± 1.0	20.7 ± 0.8
4	21.1 ± 1.2	18.8 ± 1.0	--	20.0 ± 0.8
5	20.0 ± 0.9	18.3 ± 1.1	LVF*	19.2 ± 0.7
6	21.1 ± 1.0	17.6 ± 1.2	23.0 ± 1.2	20.7 ± 0.7
7	17.8 ± 0.9	13.6 ± 1.1	23.0 ± 1.0	19.0 ± 0.8
8	20.9 ± 1.1	16.9 ± 1.3	22.0 ± 1.0	20.9 ± 0.7
9	--	19.6 ± 1.2	--	--
10	19.4 ± 1.3	20.1 ± 1.3	23.3 ± 0.9	21.0 ± 0.7
11	19.1 ± 1.1	LVF	21.7 ± 1.0	30.2 ± 1.2
12	20.7 ± 0.9	22.0 ± 1.3	19.3 ± 1.1	20.6 ± 0.7
13	17.1 ± 1.1	23.3 ± 1.0	21.0 ± 1.1	20.8 ± 0.7
14	20.0 ± 1.1	21.9 ± 0.9	20.9 ± 0.8	20.9 ± 0.5
15	19.9 ± 1.1	LVF	22.1 ± 1.3	30.5 ± 1.1
16	21.5 ± 1.1	20.3 ± 1.2	22.6 ± 1.3	21.5 ± 0.7
17	--	LVF	23.0 ± 1.2	34.3 ± 1.1
18	23.1 ± 1.0	LVF	14.3 ± 1.0	30.4 ± 1.3
19	16.5 ± 1.2	--	20.0 ± 0.9	18.4 ± 0.7
20	17.5 ± 1.2	21.7 ± 0.9		19.4 ± 0.7
21	--	20.5 ± 0.9‡		
22	22.4 ± 1.1	20.8 ± 0.8‡		
23	22.7 ± 1.0			
24	21.0 ± 1.3			
25	20.2 ± 1.3			
94		22.2 ± 1.4‡		

* Low Voltage Fast

When the frequencies in three cats were grouped the range varied from 18.4 $\pm$ 0.7 to 21.5 $\pm$ 0.7 and LVF (Fig. 4). No change in per cent alpha and beta activity was found to occur throughout the period of drug administration.

The fourth cat was removed from the drug administration program after twenty-one days and showed an initial loss of appetite and fear of handling. It looked frightened when removed to A.H.F. but appeared calm within 24 hours. After 92 days the frequency of its ECoG pattern (22.2 $\pm$ 1.4) did not significantly differ from its control (18.8 $\pm$ 1.3).

Figure 5 shows segments of ECoG tracings obtained from cat #17 to show patterns during various periods. Fig. IV of the Appendix contains sample tracings of Librium®-treated cats corresponding to the values in tables 2 and 3.

Chronic Administration of Valium®

Mean frequencies (cycles per second) of ECoG tracings after injection of increasing doses levels of Valium® are shown in table 4. Doses between 1 - 2 mg. per kg. b. wt. of diazepam resulted in increased behavioral agression. A dose level of 5 mg. per kg. b. wt. resulted in a significant increase ($p \geq 0.001$) in the frequency of the wave form (Fig. 6). The cats had pronounced ataxia of the limbs and could be easily aroused. This level of chronic drug administration was maintained.

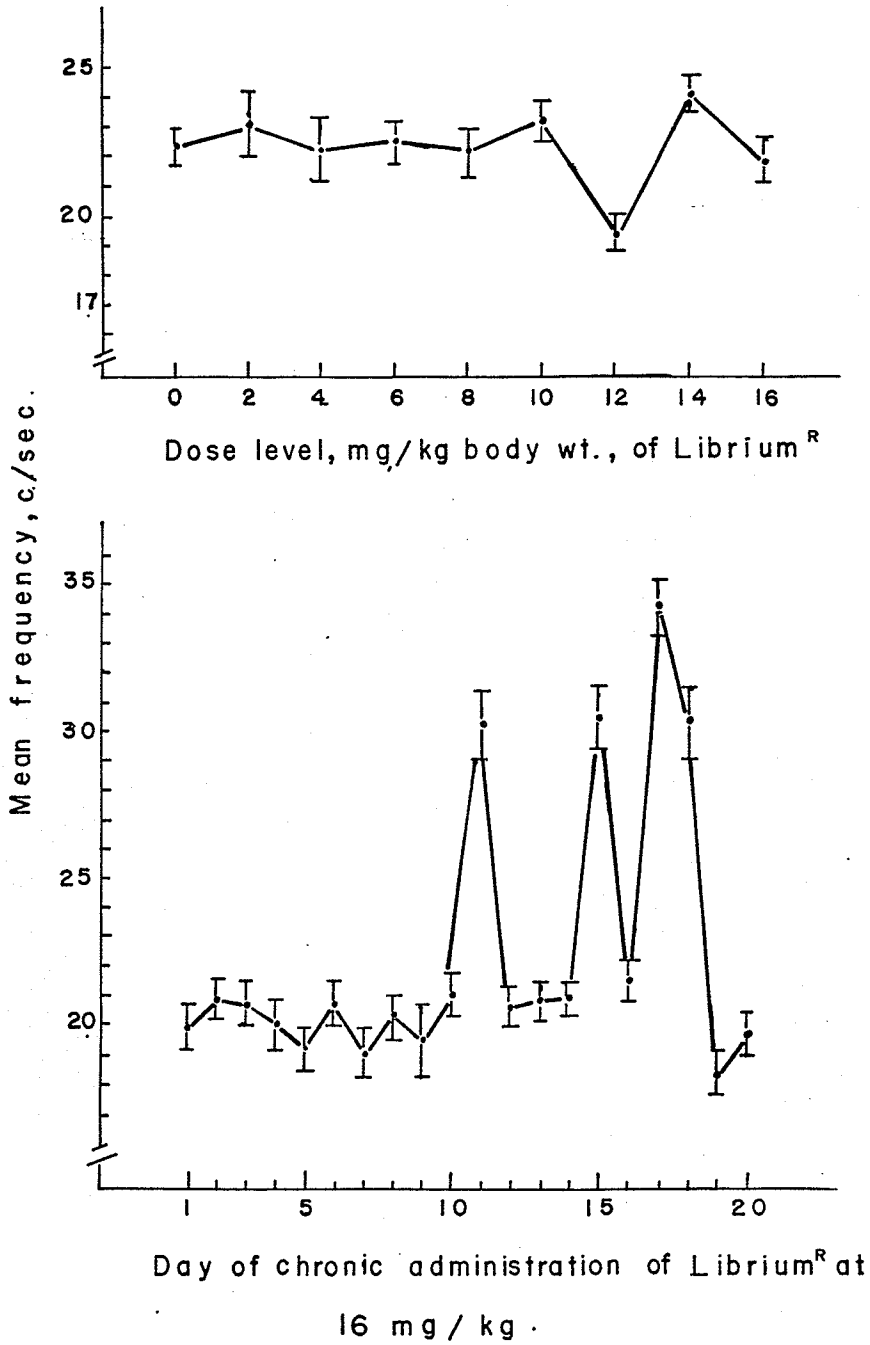


Figure 4. Mean frequencies of the ECoG after increasing and constant dose level of Librium^{R}. The upper graph shows the mean frequencies $\pm$ SE, 4 hours post- injection of increasing doses of Librium^{R}, im. The lower graph shows the mean frequencies $\pm$ SE at a constant dose level of Librium^{R}.

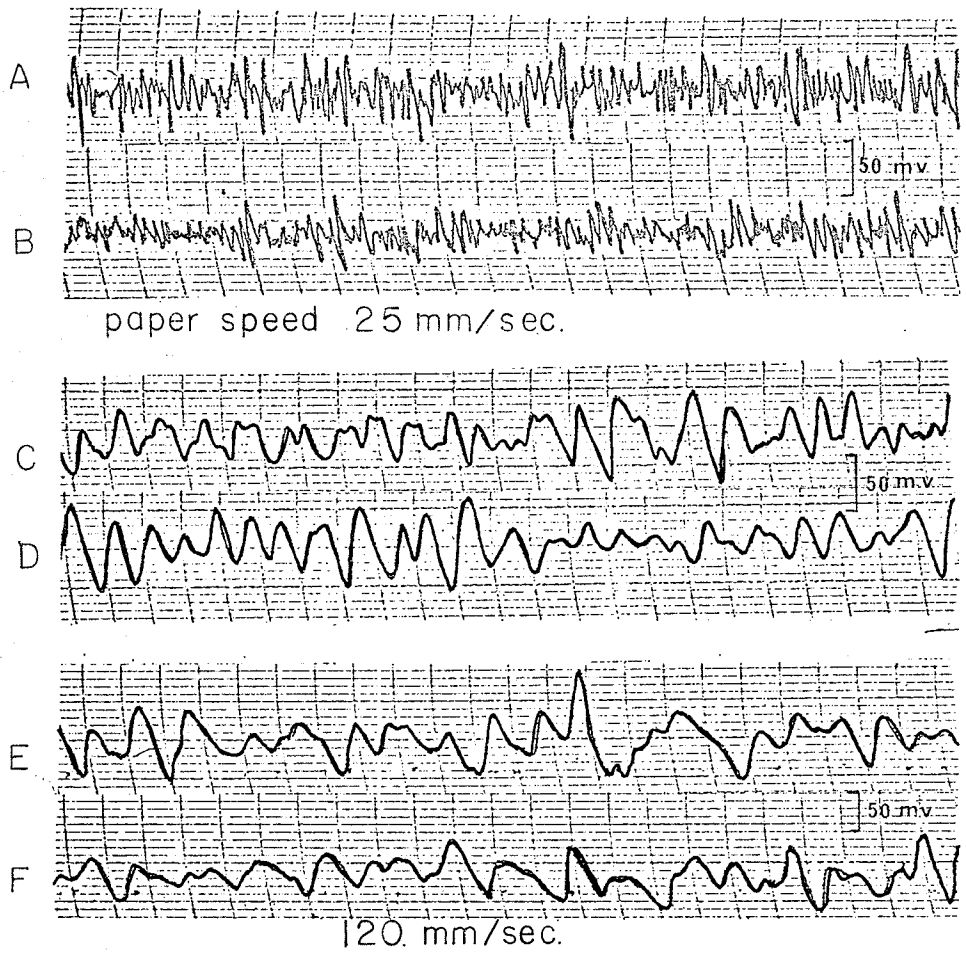


Figure 5. Sample tracings of ECoG of cat #17.

A. Predrug, no injection Mean frequency 22.9 ± 1.5

B. Predrug, saline injection 22.3 ± 1.1

C. Librium®, 4 mg. per kg. b. wt. 4 hr. p.i. 23.9 ± 1.4

D. Librium®, 10 mg. per kg. b. wt. 4 hr. p.i. 24.9 ± 1.0

E. Day 7 at 16 mg. per kg. Librium® 17.8 ± 0.9

F. Day 22 at 16 mg. per kg. 22.4 ± 1.1

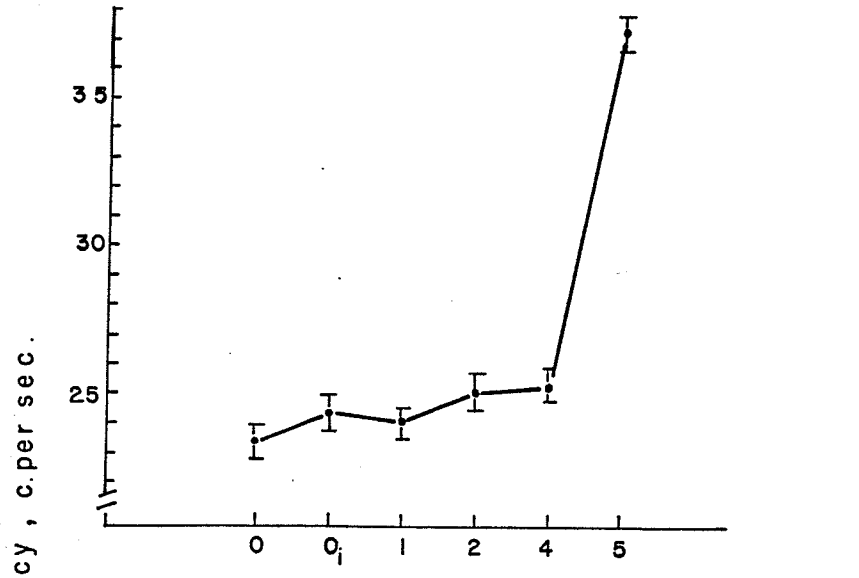
Table 4. Mean frequencies, cycles per second ± SE, of ECoG tracings at various hours after increasing doses of Valium® i.m.

Dose level mg/kg	Post inj. time hrs.	Animal Number 7	13	19	22	Grouped
1	2	23.5 ± 1.2	24.8 ± 1.4	25.3 ± 1.1	25.4 ± 0.8	24.8 ± 0.6
	4	22.6 ± 1.0	25.3 ± 1.1	24.8 ± 1.0	24.0 ± 1.1	24.1 ± 0.5
2	1	23.0 ± 1.3	27.9 ± 1.4	27.9 ± 1.1	22.0 ± 0.9	25.4 ± 0.6
	3	25.7 ± 0.8	23.1 ± 1.2	25.6 ± 1.1	25.9 ± 1.3	25.1 ± 0.6
4	2	25.4 ± 1.2	24.9 ± 1.5	27.2 ± 0.9	23.1 ± 1.1	25.3 ± 0.6
5	1	LVF*	LVF*	30.9 ± 1.4	F♯	38.3 ± 0.4
	3	LVF*	LVF*	26.4 ± 1.4	F♯	37.3 ± 0.5

* Low voltage fast activity (Over 40 cycles per second)

♯ Fast activity (Over 40 cycles per second)

Figure 6. Mean frequencies of the ECoG after increasing and constant dose level of Valium®. The upper graph shows the mean frequencies ± SE, three hours post-injection of increasing doses of Valium® im. The lower graph shows the mean frequencies at a constant dose level of 5 mg. per kg. b. wt. Valium® i.p.


35
30
25
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1
2
4
5
Mean frequency, c.per sec.
Dose level, mg/kg body wt., of Valium R

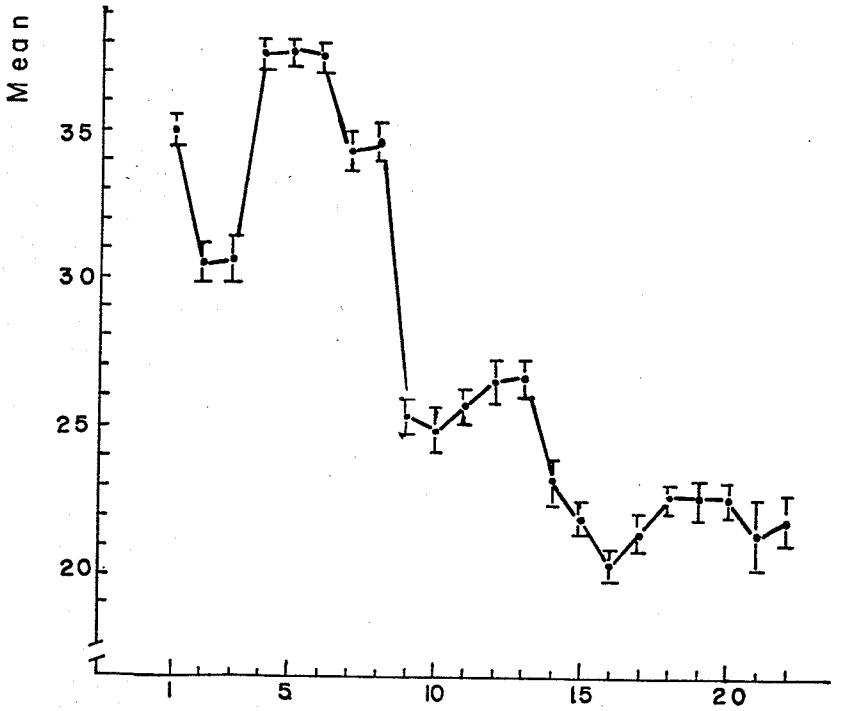
35
30
25
20
1
5
10
15
20
Day of chronic administration of Valium R at
5 mg/kg.

Table 5 shows the mean frequencies (cycles per second) of ECoG recordings kept at a constant dose level of 5 mg. per kg. b. wt. The frequencies in cycles per second ranged from:

18.8 ± 0.8 to 32.6 ± 1.2 and LVF in cat #17;

18.9 ± 1.5 to 27.0 ± 1.7 and LVF in cat #13;

22.0 ± 0.9 to 30.7 ± 1.2 in cat #19;

18.4 ± 1.1 to 26.3 ± 1.5 and F in cat #22;

20.3 ± 0.5 to 26.5 ± 0.7 and LVF when grouped for above cats. A trend towards a decrease in frequency was noted to occur in all cats after day nine. No change in behavior of the cats was observed.

Figure 7 shows the change in per cent alpha and beta activity over the 21 day period. A trend towards and increase in slower frequencies was noted.

Cat #13 was removed to AHF for 45 days and showed no observable signs of behavioral changes. It purred when handled within one week of the drug being discontinued.

Sample segments of ECoG recordings are shown in Figure 8. Additional segments of ECoG recordings are found in Fig. V of the Appendix.

Table 5. Mean frequencies, cycles per second ± SE, of ECoG recordings at a constant dose level of 5 mg per kg. b. wt. of Valium® i.p. Readings are 3 hours post-injection. Those designated "‡" are after drug was discontinued.

Day	Cat #7	Cat #13	Cat #19	Cat #22	Grouped
1	32.6 ± 1.2	F**	30.7 ± 1.2	F**	35.0 ± 0.7
2	27.2 ± 1.3	24.2 ± 0.9	26.3 ± 0.8	F	30.5 ± 0.7
3	24.3 ± 1.2	22.6 ± 1.3	28.2 ± 1.2	F	30.6 ± 0.8
4	LVF*	LVF	25.5 ± 1.4	F	37.7 ± 0.5
5	LVF	LVF	26.9 ± 1.2	F	37.7 ± 0.5
6	LVF	F	24.5 ± 1.0	F	37.5 ± 0.5
7	LVF	21.7 ± 1.3	27.7 ± 1.0	F	34.3 ± 0.7
8	LVF	27.0 ± 1.7	23.9 ± 1.2	F	34.6 ± 0.7
9	25.0 ± 1.1	24.0 ± 1.2	26.2 ± 0.8	26.3 ± 1.5	25.3 ± 0.6
10	26.3 ± 1.2	23.1 ± 1.4	25.3 ± 1.0	26.1 ± 1.6	25.0 ± 0.8
11	26.9 ± 0.9	21.8 ± 1.1	27.3 ± 0.9	26.0 ± 1.7	25.7 ± 0.6
12	31.0 ± 1.2	25.1 ± 1.1	23.2 ± 1.3	23.1 ± 1.8	26.5 ± 0.7
13	24.2 ± 0.8	26.8 ± 1.4	26.2 ± 0.9	25.0 ± 1.5	25.6 ± 0.6
14	25.8 ± 1.2	23.2 ± 1.4	23.2 ± 0.7	19.8 ± 1.6	23.2 ± 0.7
15	24.0 ± 1.4	20.5 ± 1.1	27.1 ± 1.4	25.1 ± 1.5	21.9 ± 0.6
16	23.6 ± 0.9	22.4 ± 1.7	22.6 ± 0.9	18.4 ± 1.1	20.3 ± 0.5
17	18.4 ± 0.8	20.7 ± 1.0	22.0 ± 0.9	19.8 ± 1.3	21.4 ± 0.7
18	20.1 ± 1.4	18.9 ± 1.5	25.3 ± 0.9	21.0 ± 1.5	22.6 ± 0.6
19	21.6 ± 1.0	20.0 ± 1.1	24.2 ± 1.0	23.8 ± 1.1	22.6 ± 0.6
20	22.0 ± 1.1	21.6 ± 1.1	24.9 ± 0.7	21.6 ± 2.0	22.6 ± 0.6
21	21.5 ± 1.8	19.5 ± 1.7‡		21.3 ± 1.7	21.4 ± 1.2
22	20.9 ± 1.1	23.5 ± 1.2‡		22.6 ± 1.4	21.8 ± 0.9
23		22.2 ± 1.1‡		22.0 ± 1.2	
66		22.1 ± 1.9‡			

* Low voltage fast activity (over 40 c/sec.).

** Fast activity (over 40 c/sec.).

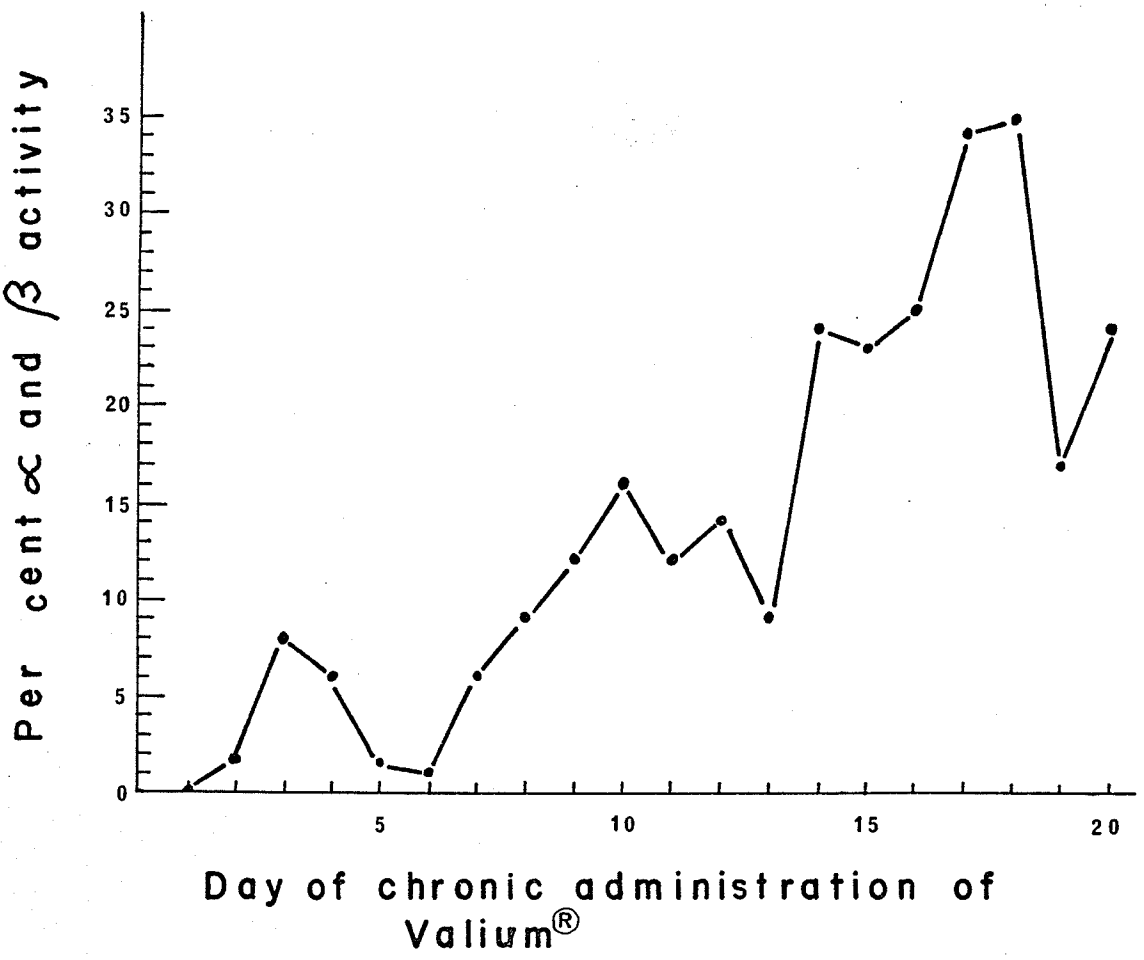

Figure 7. Change in per cent alpha and beta wave frequencies with chronic administration of 5 mg. per kg. Valium^{R}.

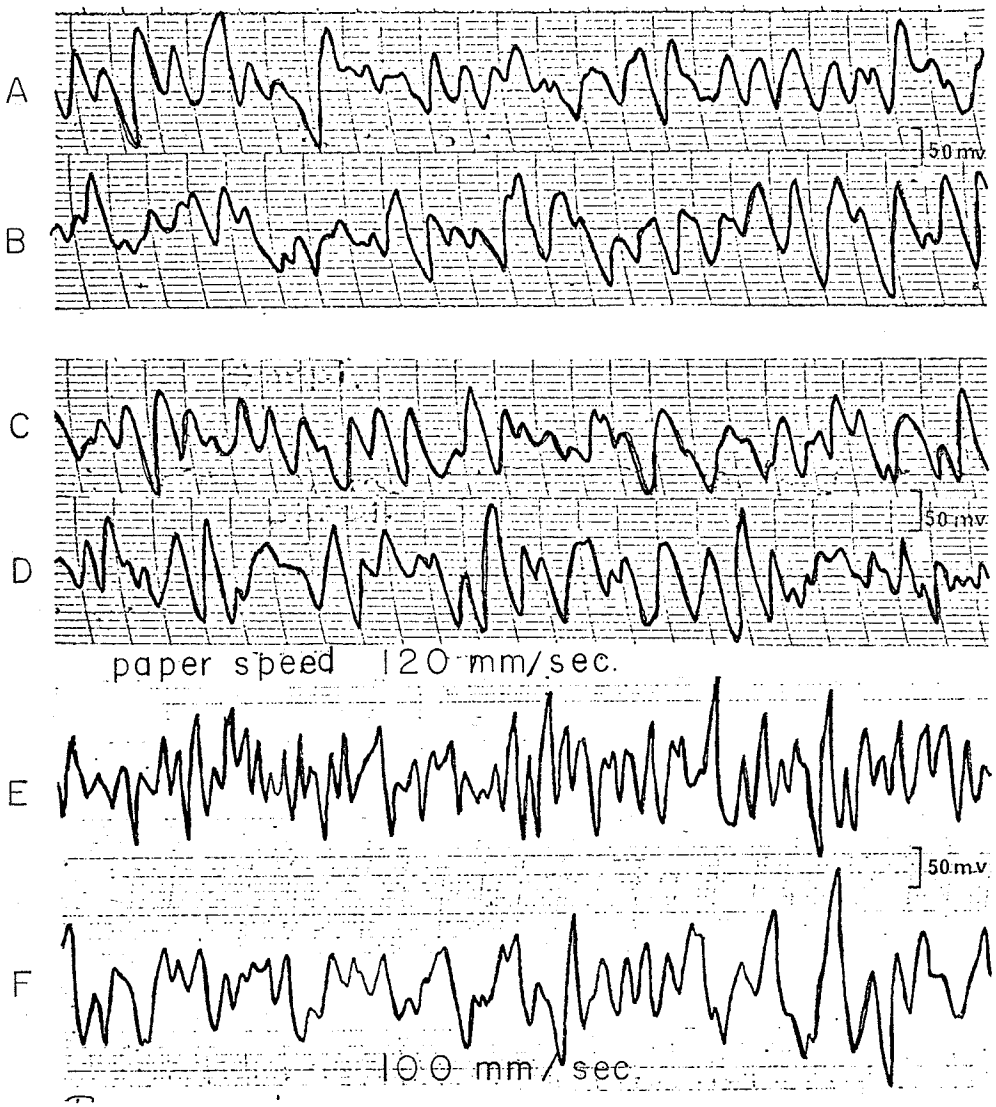

Figure 8. Sample tracings of ECoG of cat #22.

A.	Predrug, no injection	25.1 ± 0.8 c/sec.
B.	Predrug, saline injection	24.1 ± 1.0
C.	Valium®, 1 mg. per kg. im. 4 hr p.i.	24.0 ± 1.1
D.	Valium®, 2 mg. per kg. im. 3 hr p.i.	25.9 ± 1.3
E.	Day 2 at 5 mg. per kg. i.p.	Fast activity
F.	Day 22 at 5 mg. per kg. i.p.	22.6 ± 1.4

POST - MORTEM EXAMINATION

Gross Observations

The sedated cats which were killed by an overdose of Nembutal® did not go through a period of overexcitation. No infection was observed to occur in the muscle or under the Acrylic cement or silicone sheeting. Fibrous adhesions had formed between the silicone sheeting and the pia mater. The sheeting with the electrodes made a slight physical depression in the cortex. On gross observation the cortex did not appear damaged and the pial blood supply appeared intact, with no evidence of intracranial haemmorhage. Figure 9 shows the sites of the sheeting with leads which generally remained as originally placed.

Valium®-treated cats were autopsied for abdominal examination. Large lumps of fat with encapsulated pus vesicles were found to exist in the abdominal cavities with little evidence of haemmorhage.

Histological analyses

Coronal sections from the various gyri were not uniformly impregnated with silver. The upper cortical half of the sulcal area of each gyrus was best impregnated and therefore chosen for histological analysis. Impregnation of

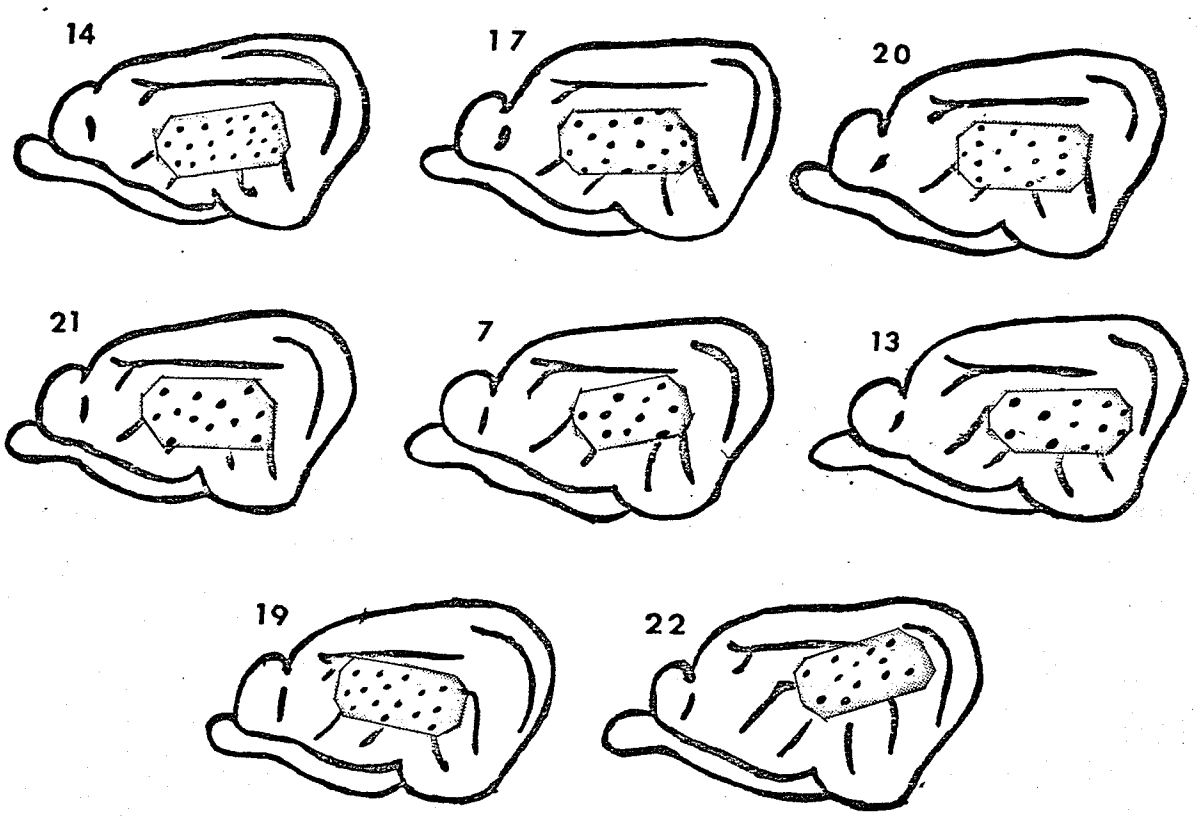

Figure 9. Areas of physical depression of the cortex due to the silicone sheeting and electrodes (Lateral view of cat cerebral hemispheres). Cats 14, 17, 20, 21 were given Librium®; cats 7, 13, 19, 22 were given Valium®.

neurons from the lower cortical half was seldom found to occur (Fig. 10).

Impregnated neurons appeared black against a yellowish-black background. The dendritic spines appeared in a variety of forms, either bent, curved or straight (Fig. 11). There was no evidence that selective staining of spines had occurred within a single dendritic tree.

Mean spine densities of apical dendrites were calculated for each of seven different functional areas (Fig. 2). The values were grouped from left gyri and their homotopes in three cats used as controls. The greatest spine density in all seven functional areas was consistantly found to occur in the branching zone (0.45 ± 0.02 to 0.63 ± 0.03), the smallest density in the stem zone (0.22 ± 0.02 to 0.30 ± 0.04), while the terminal zone had an intermediate spine density (0.30 ± 0.02 to 0.50 ± 0.06).

Stem zones of apical dendrites. Mean spine densities of stem zone dendrites were calculated in seven functional areas (Table 6 and Fig. 12). No significant change was found to occur after treatment of Valium® when compared to control values. A significant decrease in spine density was found to occur only in the marginal gyrus (VCI) following treatment of Librium®; control 0.26 ± 0.02, drug 0.20 ± 0.01 ($p \geq 0.05$).

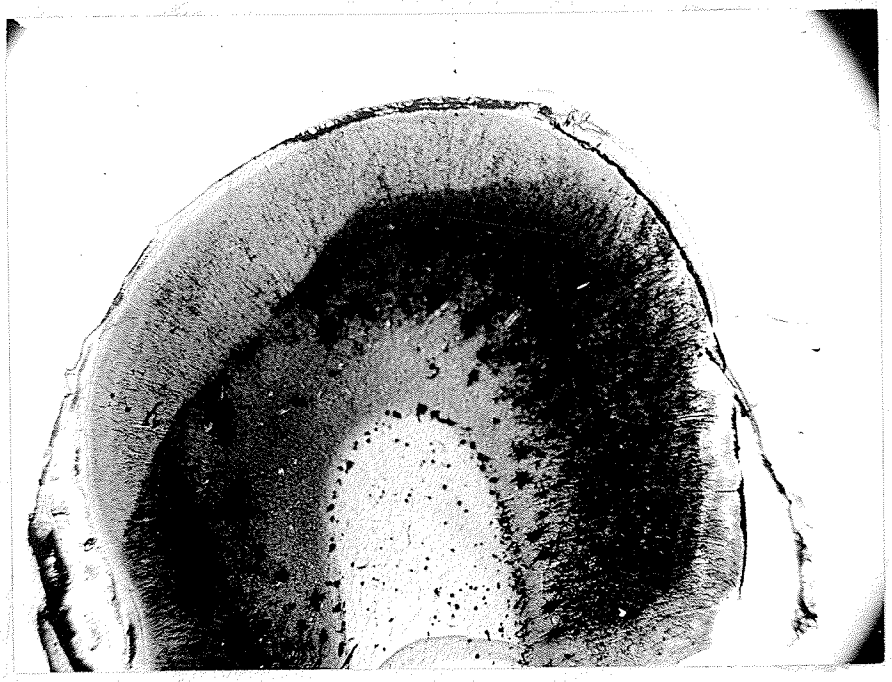

Figure 10. Photomicrograph of the posterior gyrus to show the irregular impregnation of the cortical neurons by the silver (X 45).

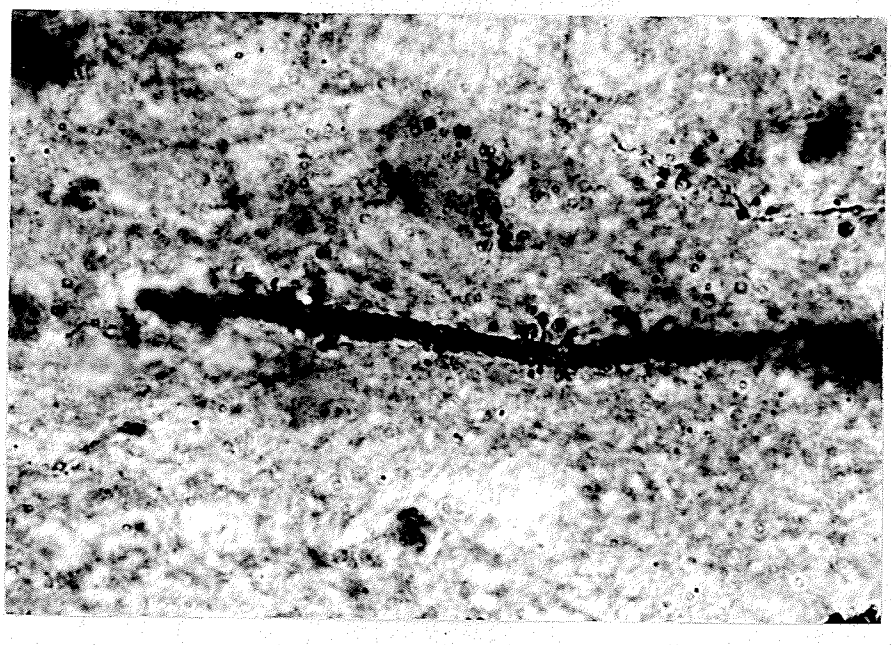

Figure 11. Dendritic spines of the branch zone of the apical dendrite from the posterior sigmoid gyrus. (X 3000).

Table 6. Mean dendritic spine densities from stem zones of apical dendrites from 6 gyri of each functional area.

Gyrus	Control	Librium® treated	Valium® treated
MSS	0.24 ± 0.04	0.21 ± 0.02	0.22 ± 0.02
MES	0.26 ± 0.02	0.28 ± 0.02	0.22 ± 0.02
PES	0.30 ± 0.04	0.32 ± 0.03	0.30 ± 0.03
CO	0.25 ± 0.02	0.23 ± 0.02	0.22 ± 0.02
PS	0.22 ± 0.02	0.22 ± 0.01	0.22 ± 0.02
VC I	0.26 ± 0.02	0.20 ± 0.01*	0.28 ± 0.03
VC II	0.23 ± 0.02	0.20 ± 0.01	0.19 ± 0.01

* Significant decrease, $p \geq 0.05$.

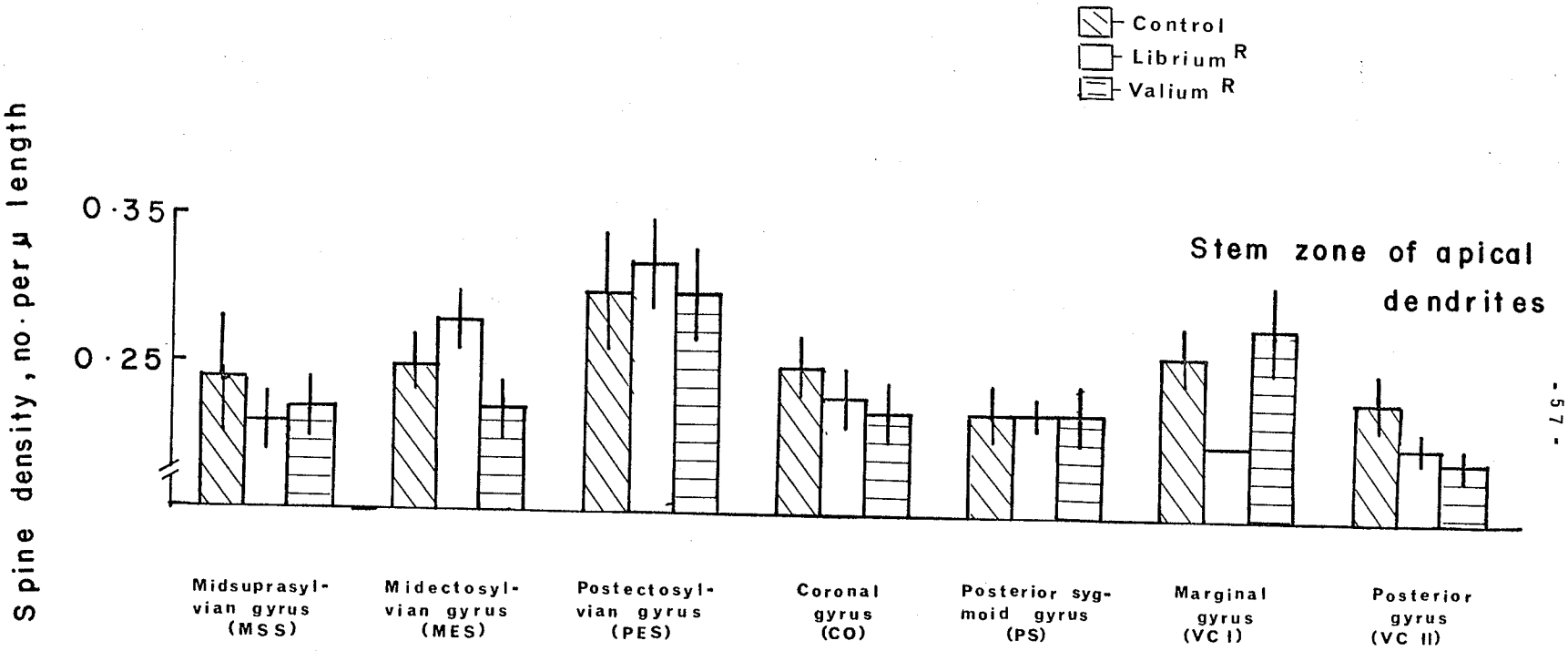

Figure 12. Mean dendritic spine densities of stem zone of apical dendrites. Oblique shading notes controls, clear bars are Librium^{R}-treated and bars with horizontal shading are Valium^{R}-treated.

Branch zones of apical dendrites. Mean spine densities of branch zones were calculated and are shown in table 7. A significant decrease in spine density was found to occur in the midectosylvian gyrus (MES) after Librium® (0.46 ± 0.02, $p \geq 0.025$) and after Valium® (0.56 ± 0.03, $p \geq 0.05$). When compared to the control value (0.63 ± 0.03).

Significant increases, $p \geq 0.05$, were noted to occur in the postectosylvian, (PES) coronal (CO) and posterior sigmoid (PS) gyri after chronic administration of Librium®. The spine densities were 0.73 ± 0.05, 0.62 ± 0.03, 0.57 ± 0.02 respectively as compared to control values of 0.59 ± 0.05, 0.53 ± 0.04, 0.49 ± 0.03.

Chronic administration of Valium® resulted in significant increases, $p \geq 0.01$, in spine densities of the postectosylvian gyrus (PES) (0.83 ± 0.03 compared to a control of 0.59 ± 0.05) and the marginal gyrus (VC I) (0.61 ± 0.02 compared to a control of 0.45 ± 0.02). There was a significant increase, $p \geq 0.05$, in the spine density of the posterior gyrus (VC II) after Valium®. The density increased to 0.69 ± 0.03 from a control of 0.50 ± 0.07. These results are shown in histogram form in Fig. 13.

Histologically impregnated dendritic spines are shown in Fig. 14. The upper photomicrograph was taken from the

Table 7. Mean spine densities from branch zones of apical dendrites from 6 gyri of each functional area.

Gyrus	Control	Librium^{R}-treated	Valium^{R}-treated
MSS	0.59 ± 0.06	0.54 ± 0.02	0.68 ± 0.03
MES	0.63 ± 0.03	0.46 ± 0.02**	0.56 ± 0.03*
PES	0.59 ± 0.05	0.73 ± 0.05‡	0.83 ± 0.03‡‡
CO	0.53 ± 0.04	0.62 ± 0.03‡	0.60 ± 0.03
PS	0.49 ± 0.03	0.57 ± 0.02‡	0.50 ± 0.02
VC I	0.45 ± 0.02	0.49 ± 0.02	0.61 ± 0.02‡‡
VC II	0.50 ± 0.07	0.47 ± 0.03	0.69 ± 0.03‡

** Significant decrease, $p \geq 0.025$, over control
* Significant decrease, $p \geq 0.05$, over control
‡ Significant increase, $p \geq 0.05$, over control
‡‡ Significant increase, $p \geq 0.01$, over control

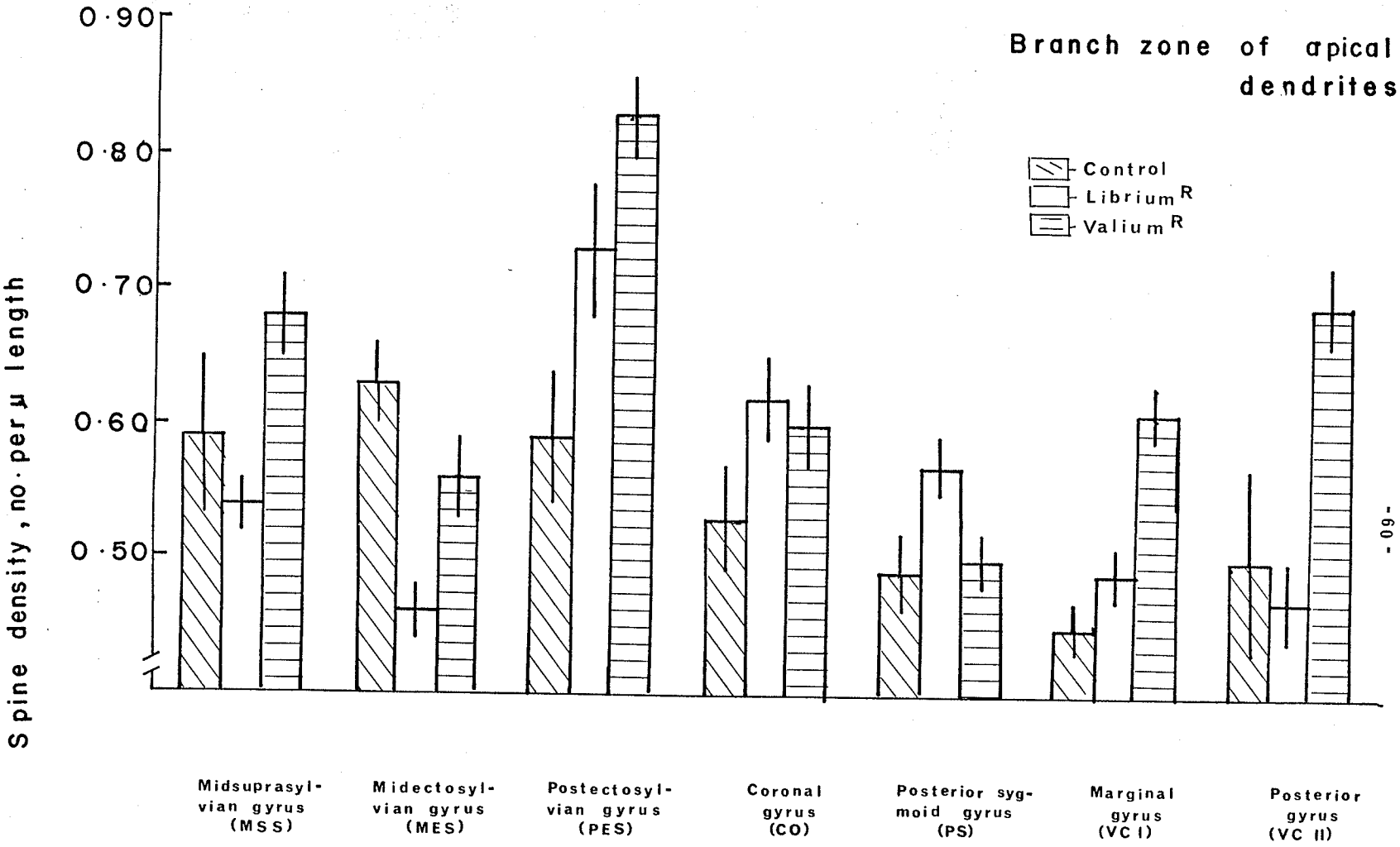

Figure 13. Mean dendritic spine densities of branch zones of apical dendrites. Oblique shading notes controls, clear bars are Librium^{R}-treated and bars with horizontal shading are Valium^{R}-treated.

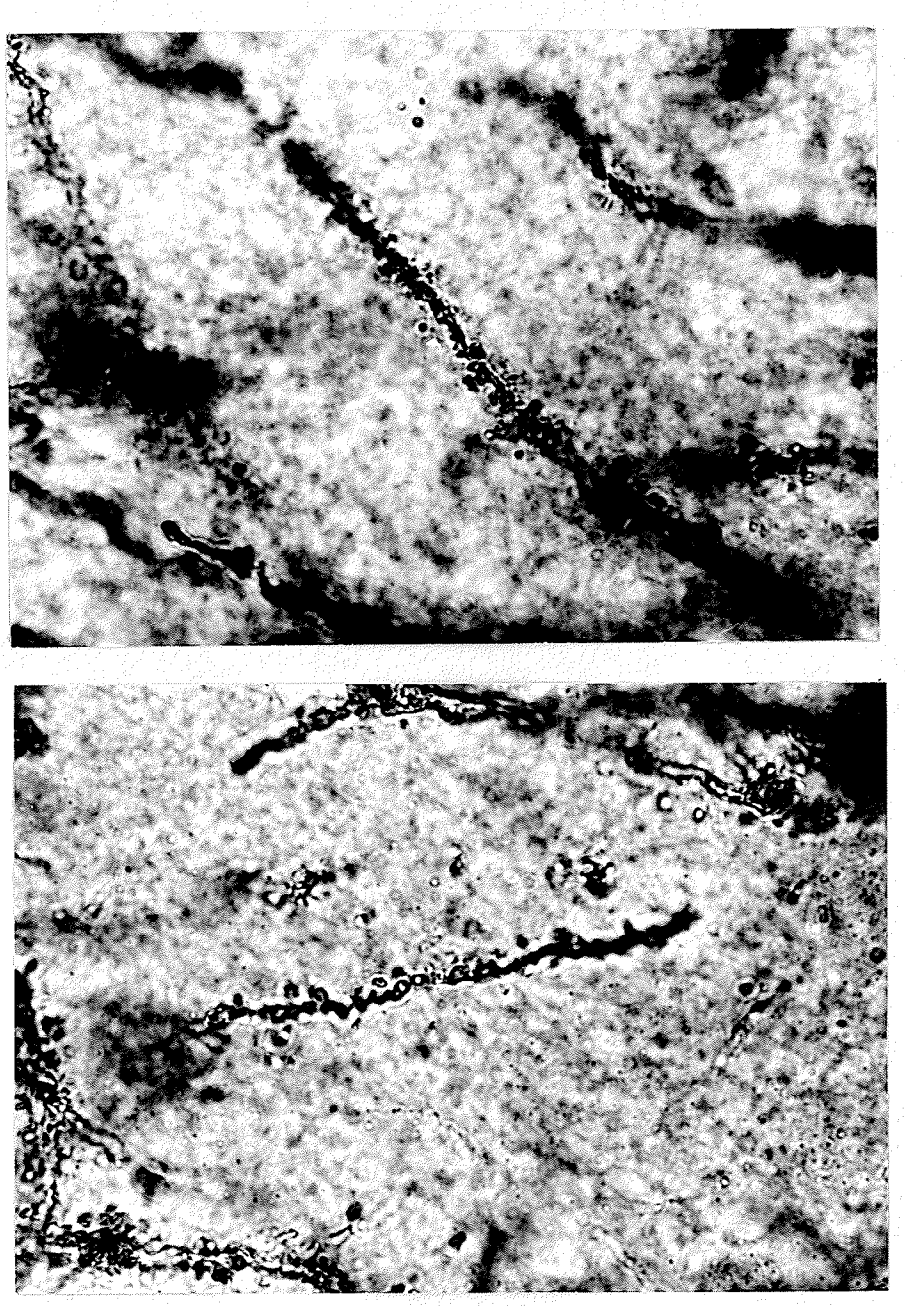

Figure 14. Photomicrographs of spines of apical dendrites. The upper photo illustrates spines of a relatively high density from the PES. The lower photo illustrates spines of a relatively low density from the MES (X 3000).

branch zone of a pyramidal neuron of the posterior ectosylvian gyrus of a Valium^{R}-treated cat. The lower photomicrograph shows dendritic spines of the branch zone from the midectosylvian gyrus after chronic administration of Librium^{R}.

Terminal zones of apical dendrites. Mean dendritic spines densities in terminal zones are shown in table 8. The midectosylvian gyrus (MES) had a significant decrease in spine density after Librium^{R} administration ($p \geq 0.025$). The density decreased from a control value of 0.50 ± 0.06 to 0.36 ± 0.03. Significant decreases were also noted to occur in the posterior gyrus (VC II) after Librium^{R} (control value of 0.39 ± 0.03 to 0.30 ± 0.02). Significant decreases were also noted in the posterior sigmoid gyrus (PS) after Valium^{R} (0.29 ± 0.01) and Librium^{R} (0.30 ± 0.02) when compared to the control value (0.36 ± 0.02).

Significant increases, $p \geq 0.05$, in spine densities occurred after Librium^{R} in the postectosylvian gyrus (PES) (0.68 ± 0.10 as compared to control value of 0.37 ± 0.04) and after Valium^{R} in the midsuprasylvian gyrus (MSS) (0.37 ± 0.02 compared to control value of 0.32 ± 0.02). Significant increases, $p \geq 0.025$, in spine densities occurred after Librium^{R} in the midsuprasylvian gyrus (MSS) (0.37 ± 0.02 from

Table 8. Mean dendritic spine densities from terminal zones of apical dendrites from 6 gyri of each functional area.

Gyrus	Control	Librium®-treated	Valium®-treated
MSS	0.32 ± 0.02	0.37 ± 0.02 ‡‡	0.37 ± 0.02 ‡
MES	0.50 ± 0.06	0.36 ± 0.03**	0.42 ± 0.01
PES	0.37 ± 0.04	0.68 ± 0.10 ‡	0.72 ± 0.06 ‡‡
CO	0.30 ± 0.02	0.33 ± 0.02	0.39 ± 0.02 ‡‡
PS	0.36 ± 0.02	0.30 ± 0.02**	0.29 ± 0.01**
VC I	0.32 ± 0.02	0.35 ± 0.02	0.39 ± 0.01 ‡‡
VC II	0.39 ± 0.03	0.30 ± 0.02**	0.42 ± 0.02

** Significant decrease, $p \geq 0.025$, over controls
‡ Significant increase, $p \geq 0.05$, over control
‡‡ Significant increase, $p \geq 0.025$, over control

control value of 0.32 ± 0.02) and after Valium® in the postectosylvian gyrus (PES) (0.72 ± 0.04 from 0.37 ± 0.04), coronal gyrus (CO) (0.39 ± 0.02 from 0.30 ± 0.02 from 0.30 ± 0.02), and the marginal gyrus (VC I) (0.39 ± 0.01 from 0.32 ± 0.02). These results are shown in histogram form in Fig. 15.

Basilar dendrites. Basilar dendrites of all seven cortical areas had mean spine densities ranging from 0.16 ± 0.02 to 0.25 ± 0.05 (Table 9). The mean dendritic spine density significantly decreased, $p \geq 0.025$, in the midecto-sylvian gyrus (MES) (0.18 ± 0.02) after Librium® when compared to the control value (0.23 ± 0.02). These results are shown in histogram form in Fig. 16.

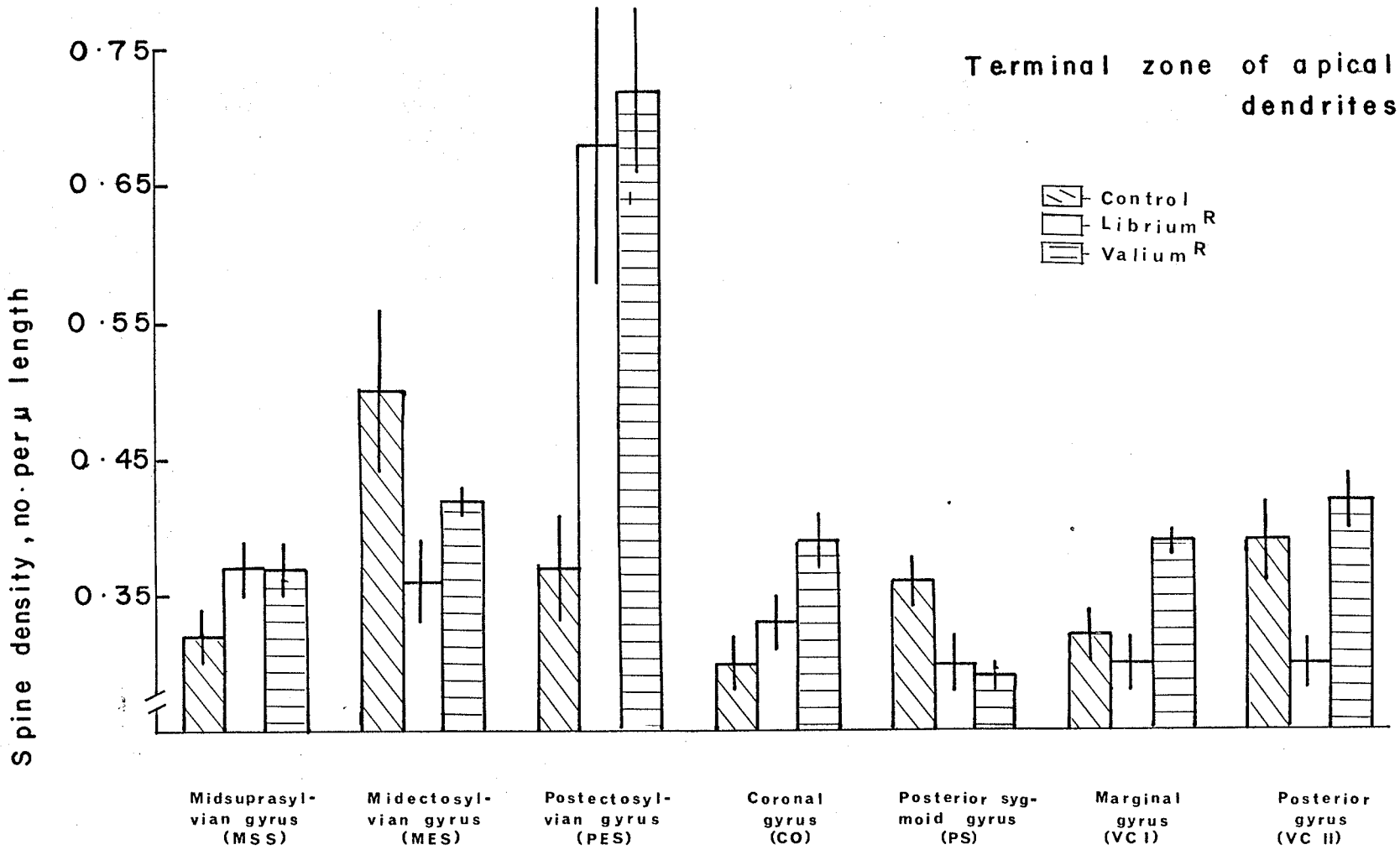

Figure 15. Mean dendritic spine densities of terminal zones of apical dendrites. Oblique shading notes controls, clear bars and Librium^{R}-treated and bars with horizontal shading are Valium^{R}-treated.

Table 9. Mean dendritic spine densities from basilar dendrites from 6 gyri of each functional area.

Gyrus	Control	Librium®-treated	Valium®-treated
MSS	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.02
MES	0.23 ± 0.02	0.18 ± 0.02**	0.20 ± 0.02
PES	0.22 ± 0.02	0.21 ± 0.01	0.25 ± 0.02
CO	0.22 ± 0.01	0.21 ± 0.02	0.25 ± 0.02
PS	0.20 ± 0.02	0.21 ± 0.03	0.22 ± 0.03
VC I	0.16 ± 0.02	0.19 ± 0.02	0.19 ± 0.02
VC II	0.21 ± 0.03	0.20 ± 0.03	0.25 ± 0.05

** Significant decrease, $p \geq 0.025$, over control

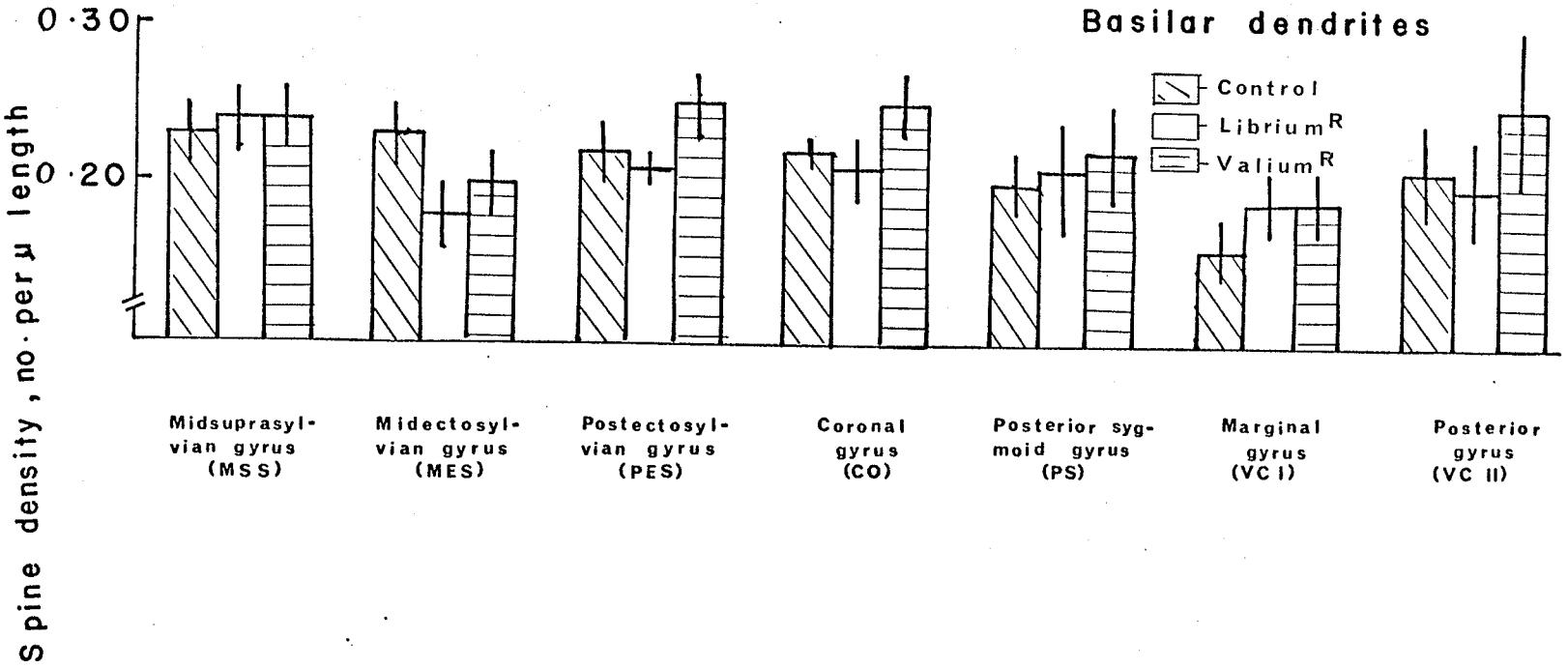

Figure 16. Mean dendritic spine densities of basilar dendrites. Oblique shading is for controls, clear bars are Librium^{R}-treated and bars with horizontal shading are Valium^{R}-treated.

DISCUSSION

Cats are well suited by reason of their apparent resistance to mass infection to be experimental animals in a chronic study of this type. The insertion of pial electrodes did not affect the apparent health of each cat. A slight, localised infection around the plug-skin junction did not appear to alter the gross behavior of the cat or the electrical activity of the cerebral cortex. The abdominal infection seen in the diazepam-treated cats was also localised and appeared to cause some discomfort without apparent pyretogenesis since autopsy examination indicated that the infection was encapsulated. It would have been beneficial to have measured pulse rate and blood pressure throughout this study, but it was not possible.

Analyses of Electrical Activity

ECoG tracings are a valid means of monitoring the generalised spontaneous electrical activity of the cerebral cortex. Subjective behavioral observations of cats were maintained throughout this study in the hope that behavioral changes before and during drug administration could be correlated to changes of the ECoG pattern. Observations of the cat's behavior and the ECoG values found in this study

under control conditions, 18.8 to 26.7 cycles per sec., are comparable to those previously reported by Kido et al. (1966) from pial electrodes on the surface of the anterior sigmoid gyrus. They reported that excited cats which show behavior attention reactions, sensory and motor, have frequencies of 40 to 45 cycles per second. Cats behaviorally awake, with no behavior attention reactions, exhibit frequencies of 20 to 30 cycles per sec. Cats behaviroally at rest sit or lie in a supine position with legs curled under their bodies and have corresponding ECoG frequencies of 10 to 20 cycles per sec. Frequencies of 6 to 8 or 10 to 12 cycles per sec. are found in cats which are drowsy and have behavioral states similar to that of cats at rest. Cats that exhibit deep sleep are completely relaxed with an ECoG showing prominent δ rhythms with spindle bursts. Activated sleep, characterised behaviorally by twitches of ears, vibrissae and extremities, is characterised electrically by prominent fast activity.

In this study the dose of Librium^{R} found to be effective in inducing sedation with pronounced ataxia was 16 mg. per kg. i.m. The behavioral responses found to occur at this level correspond to observations of behavior obtained by Kido et al. (1966). They reported that 20 mg.

per kg. p.o. of Librium® induced sedation to a level of behavioral and electrical light sleep. In the present study the lack of a significant change in the ECoG may be due to the fact that multipolar electrodes with a common average reference lead were used. It is possible that certain specific cortical areas may have shown either significant increases or decreases in electrical activity but these differences would become averaged by the use of the common average reference lead. In contrast, Kido et al. (1966) used a unipolar lead which recorded the electrical activity from a specific point source on the anterior sigmoid gyrus. A second possibility for the lack of a significant change in frequency may be that the drug acted on subcortical inhibitory centers, areas as yet undetermined, reducing transmission of inhibitory impulses to the cerebral cortex. This in turn could result in increased energy demands by the neurons, thereby causing an increased metabolism. This may explain the obvious appetite increase which could be dictated by physiological demand. Also, a psychological demand for increased appetite could have resulted directly from drug action upon centers in the lateral hypothalamus that stimulates appetite, or indirectly by blocking transmission of impulses from centers in the ventromedial hypothalamus

that inhibit appetite stimulation (Morgane, 1961; Hoebel and Teitelbaum, 1962; Kennedy, 1966).

The values from ECoG tracings obtained from Valium^{R} administration correspond to those previously reported in cats by Requin et al. (1963). In the present study, as also reported by Requin, doses of 1 to 2 mg. per kg. i.m. produced fast activity and a definite behavioral change to increased agressiveness. Doses at a level of 5 mg. per kg. i.m. and i.p. resulted in pronounced ataxia with a slight head droop and dilation of pupils. The ECoG did not indicate light sleep although behavioral reactions were typical of light to deep sleep. The decision to maintain the dose level at 5 mg. per kg. was determined on subjective observations of the cat's behavior as well as increased ECoG frequency. The significant decrease in fast activity after Day 9 may be an indication of tolerance. It is also tempting to suggest that the decrease may be due to fatigue of specific neurons which are unable to cope with the increased neuronal activity.

There is general agreement that the potency of diazepam as a muscle relaxant is three times greater than chlordiazepoxide (Randall et al., 1961; Schallek et al., 1964; 1965). The results obtained in this study from Valium^{R} at 5 mg. per kg. and Librium^{R} at 16 mg. per kg. are in agreement with these earlier findings. However a constant dose level

required to produce light sleep with pronounce ataxia in cats has not been reported in the literature. Randall et al. (1961) found that sleep was induced by Valium® at 3 mg. per kg. i.v. and by Librium® at 10 mg. per kg. i.v. Hernandez-Peon et al. (1964) found ataxia and electrical fast activity in cats after Valium® at doses of 2 to 4 mg. per kg. i.p. Banziger (1965) found sedation to a sleep level with labored breathing and pronounced ataxia occurred after oral administration of 10 mg. per kg. diazepam and 6 to 12 mg. per kg. Librium®. Schallek and Kuehn (1965) found that light sleep with easy arousal could be induced in cats with Librium® at a dose level of 10 mg. per kg. p.o. Explanations which could account for these differences have not yet been reported in the literature. Perhaps different techniques are involved which give the inconsistant results, although all studies used the commercial preparations of Librium® and Valium®. Since the results obtained in this study corroborate some of the earlier findings, the present dose values should be equally valid.

Benzyl alcohol, the drug vehicle of Valium®, has been reported recently to affect the potency of the drug (Crankshaw and Raper, 1971). Diazepam in benzyl alcohol is twelve times more potent than chlordiazepoxide in a water solvent. Potentiation of the action of chlordiazepoxide may

also occur when administered in a pharmacologically active vehicle such as propylene glycol or benzyl alcohol. It is tempting to suggest that potentiation by the drug vehicle affects the electrical activity and perhaps the spine density data in this study as drug vehicle controls were given only 0.2 cc i.m. per day.

One interesting clinical pharmacological effect of benzodiazepines was illustrated by their effectiveness as a preanaesthetic muscle relaxant (Tornetta, 1965). The cats in this study did not exhibit the characteristic initial period of excitation when an overdose of sodium pentobarbital was administered at the end of the chronic drug treatment.

It can be concluded, therefore, from this portion of the study, that ECoG tracings obtained from a common average reference lead offer adequate means of analysis of the general electrical activity although specific cortical electrical activity changes may not be monitored adequately. Many specific point source electrodes over specific areas would result in a more definite analysis of the cortical activity. It may be concluded also that the benzodiazepines do affect the spontaneous electrical activity of the cerebral cortex either by direct action or indirect action on subcortical centers such as the hypothalamus.

Analysis of Dendritic Spine Densities

Since the Golgi Rapid Method resulted in erratic staining of neurons in this study, a quantitative study of the neuronal packing density was not possible. Changes in branch densities and angulation were not calculated. Brandes (1971) found that there were no changes in branch patterns of dendrites of the lateral geniculate body after enucleation of the retina in adult cats. However he did report that corresponding decreases in spine densities of the visual cortex were found to occur. It may be conculded, therefore, that surgical deafferentation at a distal area may decrease spine densities without a corresponding decrease in dendrite branch densities.

The Golgi Rapid Method impregnates with silver only 1 1/2 to 2 per cent of the available neurons (Sholl, 1953). Spine densities were calculated from impregnated neurons in the upper cortical half as was the method of study by Sholl (1953). It is important therefore to note that relative spine densities were calculated rather than absolute values.

Slight subpial dendritic beading was seen to occur in the mid-gyral portion of some sections. No reason for this phenomenon has been reported. Such variocosities may be precursors of dendritic spines, artifacts of fixation of the

result of degeneration of the axonal input (W. Harris, 1960; Grant and Aldoskogius, 1967). Since variocosities were found only in the subpial region, it is tempting to suggest that they are the result of both degeneration by autohistolysis and the harsh treatment of fixation.

Spine densities vary in relation to their zone of the dendritic tree. Globus and Scheibel (1966; 1967d) reported that branch zones of the rabbit neocortex had a higher spine density than terminal zones. Weisman (1970) reported branch zones of apical dendrites in the association cortex (MSS) of the adult cat had a higher spine density (6.3) than the terminal zone. The terminal zone had a higher density (1.8) than the stem zone (1.2). In the present study highest densities were found also in branching zones, lowest in the stem zones and intermediate densities in the terminal zones. Differences in values may be due to discrete variations in the techniques used and subjective interpretation in the indentification of dendritic spines. Although the values differ between studies, there is general agreement that the highest density occurs in the branch zone. This was seen in each of the seven different functional areas examined in the present study. If spine density is equated with afferent input, it can be assumed that the greatest afferent input occurs in the dendritic branch zone.

Environmental manipulation of afferent input and surgical deafferentation have shown that dendritic spines may be indicators of neuronal use and disuse (cf. literature review). Chemicals also have been reported to alter neuronal structures. Diphenylhydantoin, after chronic administration to rats, induced "sprouts", laminar bodies, in dendrites of Purkinje cells of the cerebellum (Snider and delCerro, 1967). Trifluoroperazine administration to rabbits resulted in accumulation of glycogen granules in dendrites (Koizumi and Sheraishki, 1970). Schadé and Caveness (1968) reported that the transsynaptic atrophy of monkey cerebral cortical dendrites after X-irradiation was probably due to chemical alterations. In this study the alterations in spine density may be the result of the chemical action of the two benzodiazepines rather than the result of neuronal use and disuse.

The data obtained from histological analysis of the Librium®-treated cats shows a slight correlation to the ECoG patterns as reported in an earlier section of this thesis. In the total cortical area studied an equal number of increases and decreases in spine densities were found to occur. Perhaps the overall affect of these density changes resulted in the averaging of the ECoG frequencies as suggested earlier in the discussion. In respect to Valium®-treated cats

more increases than decreases were found. This correlates to the overall increase in frequency reported in the ECoG, and may be of physiological significance. The decrease in mean frequency after day nine (see Fig. 6) may have been the result of neuronal fatigue induced by the continuous fast activity. The first auditory area, over which the common average reference lead was situated, does show a decrease in spine density which may be correlated to the decrease in mean frequency.

The density of stem zone spines showed no significant change after either drug with two exceptions. A decrease in density was found to occur in the second visual area (VC I) after Librium®. Basilar dendrites in the first auditory area (MES) had a significant decrease in spine density only after Librium®. No other changes in basilar dendrites were noted. It can be concluded, therefore, that these two dendritic zones were affected very little by drug action. Reasons for this are open to speculation. Perhaps these areas can adequately compensate by synapses en passant, for any changes in afferent input or perhaps these are sites of generalised input which are not easily altered.

Major changes occurred in dendritic branch and terminal zones. Such alterations occurred in the first (MES)

and association (PES) auditory areas. Significant decreases occurred in the MES in the branch zones after both drugs and in the terminal zone after Librium®. Significant increases in both dendritic zones of PES were seen after both drugs. A possible explanation may be that the drug inhibited impulses to the MES, for reasons as yet unknown, causing decreases in spine densities. Afferent input from subcortical areas to MES may have alternate sites of input to the cortex such as the PES, which may become functional when the primary site of input is inhibited. As the MES is connected to PES by intracortical fibers, it can be suggested that neuronal disuse in the MES resulted in a functional compensatory increase of afferent input to PES, thus necessitating a corresponding increase in spines. This could also explain the increase in spine density found in the association (MSS) area terminal zones, as the MES communicates to the association area by means of intergyral fibers.

The decrease in spine density found in the terminal zone of the sensorimotor cortex (PS) after Valium® may be correlated to the loss of muscle coordination and, correspondingly, loss of input from the cerebellar cortex. However, after Librium® significant increases were seen in this gyrus in both dendritic zones although the cats exhibited similar

gross stages of ataxia. The differences in the direction of change could be a result of the differential action of the two drugs, as this was the only cortical area in which opposite effects were seen.

The deep sensory cortex (CO) showed increases in spine densities, but in a different dendritic zone with each drug. This may be the result of specific drug action on specific sites on the neuron, or alternatively could be the result of blocking specific inhibitory impulses at a sub-cortical level. The changes in visual cortical spine densities may be explained in a similar way. Librium^{R} resulted in only one density change in the terminal zone of the third auditory area (VC II). Valium^{R}-treated cats showed increases in all but the terminal zone of VC II. These increases in density could possibly be correlated to the constant dilation of the cats pupils throughout the study, resulting in an involuntary increased input to the visual cortex.

The site of action of benzodiazepines has not yet been determined (cf. literature review). However their action on cardiovascular systems (Chap and Wang, 1966) and their appetite stimulant effects indicate that the hypothalamus may be affected by presynaptic inhibition

(Stratton and Barnes, 1971).

It can be concluded, therefore, that these chemicals cause changes in dendritic spine densities. The mechanisms by which these changes in structure are effected, and the sites upon which the drugs act to effect the changes are unknown although it would appear that the benzodiazepines act both on the hypothalamus and cerebral cortical neurons.

SUMMARY

1. An indwelling electrode assembly unit was placed on the pial surface of each of eleven cats for the purpose of monitoring the spontaneous electrical activity of the cerebral hemispheres before and during chronic administration of two drugs. This experimental procedure did not affect the general health of the animals.

2. The frequency of electrical activity of the cats in predrug controls, 18.8 to 26.7 cycles per sec., indicated that their electrical activity could be correlated to their behavioral state of rest.

3. Chronic administration of chlordiazepoxide (Librium®) at 16 mg. per kg. i.m. did not significantly alter the electrical activity of the cat although expected behavioral changes occurred.

4. Chronic administration of 5 mg. per kg. i.p. of diazepam (Valium®) resulted in a significant increase in fast activity for the first nine days, followed by fast activity at a lower level. Ataxia and sedation were seen throughout the 21 days.

5. The analysis of the dendritic spines indicated that these drugs cause discrete changes in density, perhaps by acting on the hypothalamus or directly upon the dendritic

tree. This shows that chemical alteration of afferent input affects spines as does surgical deafferentation and environmental manipulation.

6. Changes in spine densities of the different cortical areas:

A) Librium®-treated.

Cortical area	Stem zone	Branch zone	Terminal z.	Basilar
Association MSS	N.S.	N.S.	Increase**	N.S.
Auditory MES	N.S.	Decrease**	Decrease**	Decrease**
PES	N.S.	Increase*	Increase*	N.S.
Deep sensory CO	N.S.	Increase*	N.S.	N.S.
Somatosensory PS	N.S.	Increase*	Increase**	N.S.
Visual VC I	Decrease**	N.S.	N.S.	N.S.
VC II	N.S.	N.S.	Decrease**	N.S.

B) Valium®-treated.

Association MSS	N.S.	N.S.	Increase*	N.S.
Auditory MES	N.S.	Decrease*	N.S.	N.S.
PES	N.S.	Increase**	Increase**	N.S.
Deep sensory CO	N.S.	N.S.	Increase**	N.S.
Somatosensory PS	N.S.	N.S.	Decrease**	N.S.
Visual VC I	N.S.	Increase**	Increase**	N.S.
VC II	N.S.	Increase *	N.S.	N.S.

* Significant $p \geq 0.05$
** Significant $p \geq 0.025$

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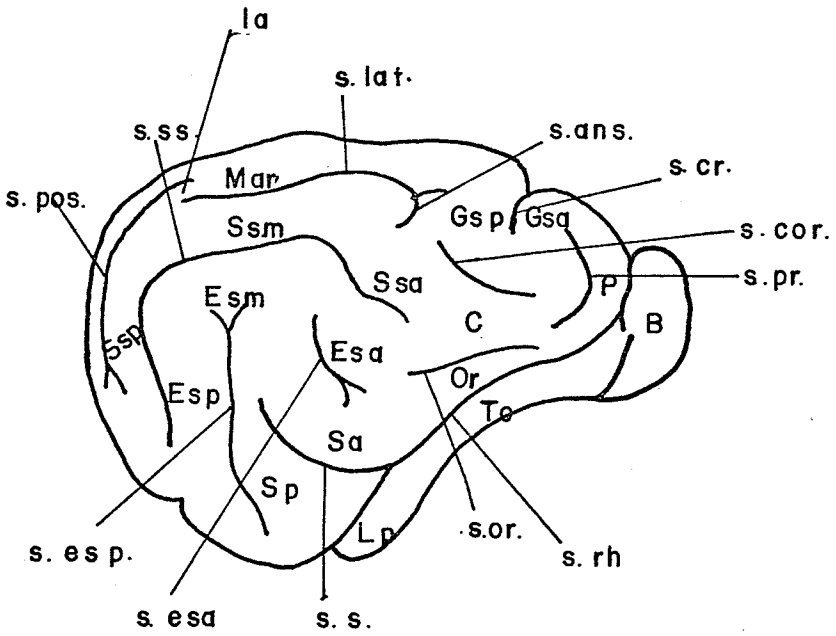
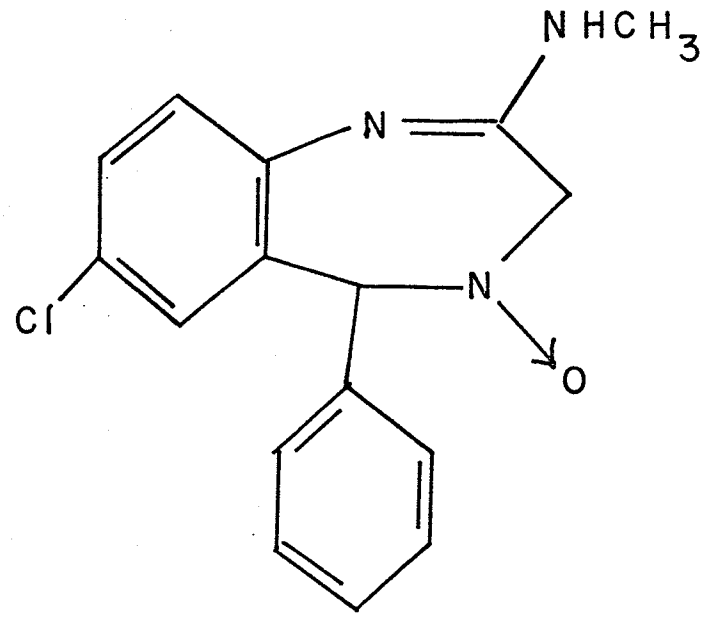
APPENDIX



Appendix. Figure I. Anatomical structures of cat cerebral hemisphere (lateral view). After Bures et al. (1967). Lp: Pyriform lobe; To: Olfactory tract; B: Olfactory bulb.

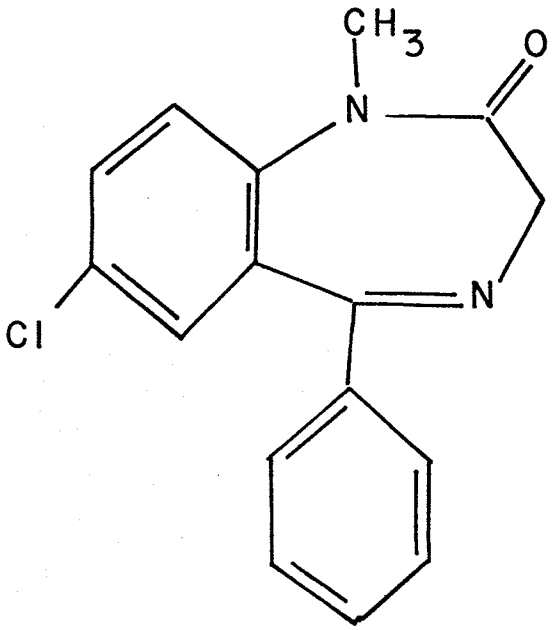
Gyri: C: coronal
Esa: anterior ectosylvian
Esm: mid ectosylvian
Esp: post ectosylvian
Gsp: post sigmoid
Gsa: anterior sigmoid
Mar: marginal
Or.: orbital
P: proreus
Sa: anterior sylvian
Sp: post sylvian
Ssa: anterior suprasylvian
Ssm: mid suprasylvian
Ssp: post suprasylvian

Sulci: s. ans: ansatus
s. cor: coronal
s. cr.: cruciate
s. esa: ant. ectosylvian
s. esp: post ectosylvian
s. lat: lateral
s. or : orbital
s. pr : presylvian
s. rh : rhinalis
s. s : sylvian
s. ss : suprasylvian
s. pos: posterior



CHLORDIAZEPOXIDE

7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide



DIAZEPAM

7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one

Appendix. Figure II. Structural formulae of chlordiazepoxide and diazepam.

Appendix. Figure III. Histological procedure from Weisman, 1970.

1. Fix in 4:1 2.5% w/v aq. Potassium dichromate ($K_2Cr_2O_1$)
   1% w/v aq. Osmium tetroxide (OsO_4)
   for 72 ± 3 hours.
2. Wash in distilled water -30 sec.
3. Wrap slab in gauze and immerse in 0.75% w/v aq. Silver nitrate ($AgNO_3$). for 1 - 2 hours.
4. Fresh 0.75% w/v $AgNO_3$ for 46 ± 2 hours - Gentle agitation.
5. 30% EtOH - 2 hours.
6. 70% EtOH - 8 hours.
7. 95% EtOH - 24 hours.
8. Absolute EtOH - 24 hours.
9. Absolute EtOH: Absolute EtOH -ether 1:1 for 24 hours
10. 1 $\frac{1}{2}$% LVN celloidin in EtOH -ether - 1 day.
11. 3% LVN celloidin - 1 day.
12. 6% LVN celloidin - 1 day.
13. Saturated LVN celloidin - 1 day.
14. Embed in Sat. LVN celloidin in paper box. Place in dessicator - 2 days.
15. Trim and block.
16. Section when hard - 40 μ sections.

17. Dehydration and clearing

a) 95% EtOH - 15 min.

b) 100% EtOH - 15 min.

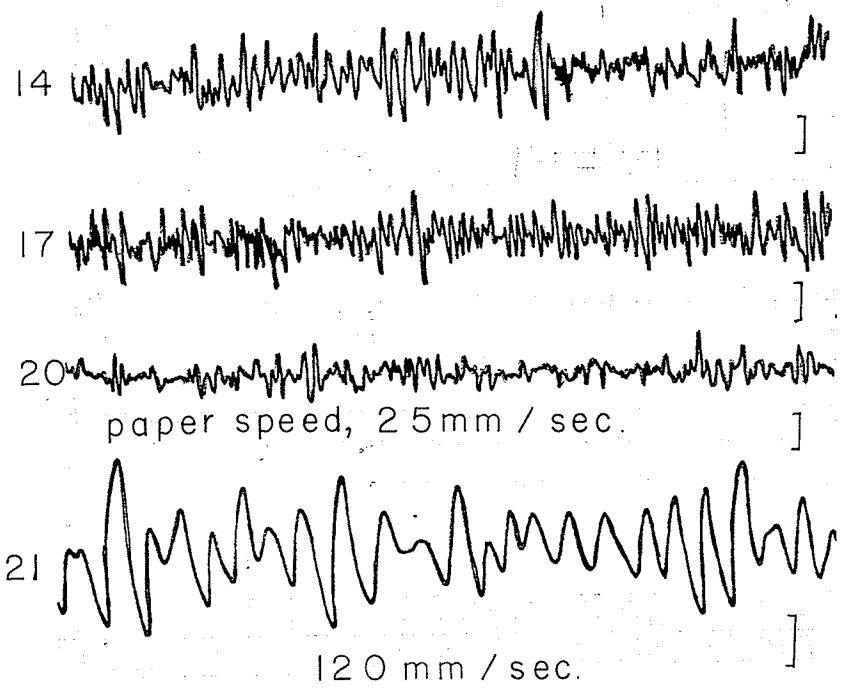
c) 1:1 Abs. EtOH: Terpineal - 15 min.

d) 100% Terpineal - 15 min.

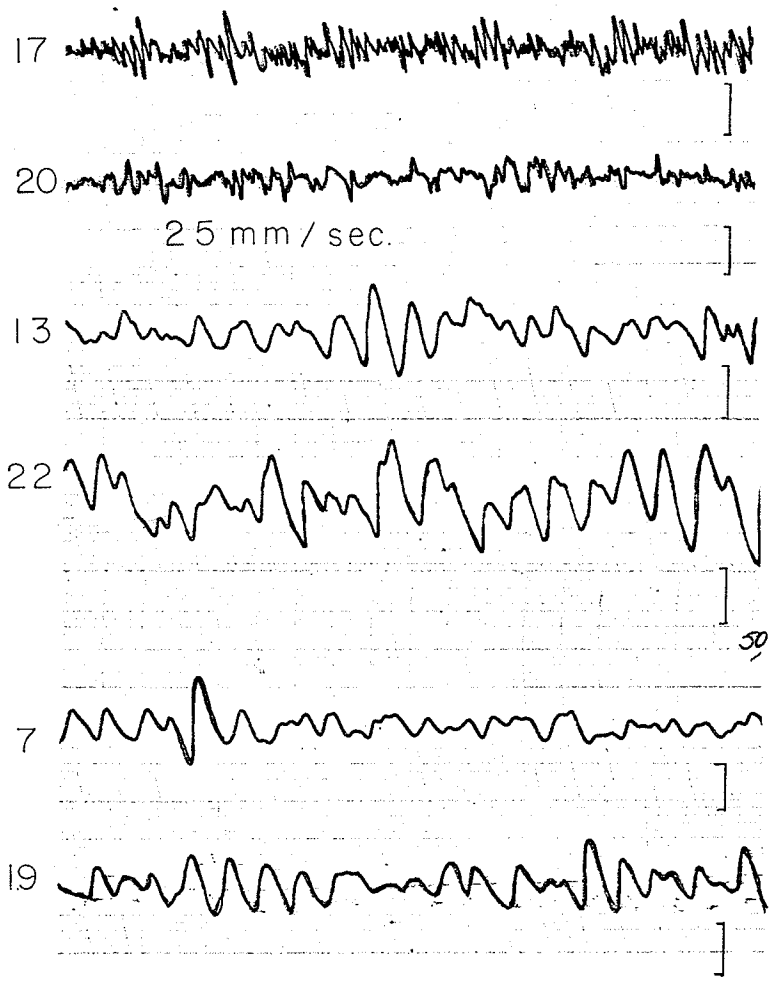
e) Mount with Permount - Cover.

f) Dry at 45° for 2 days.

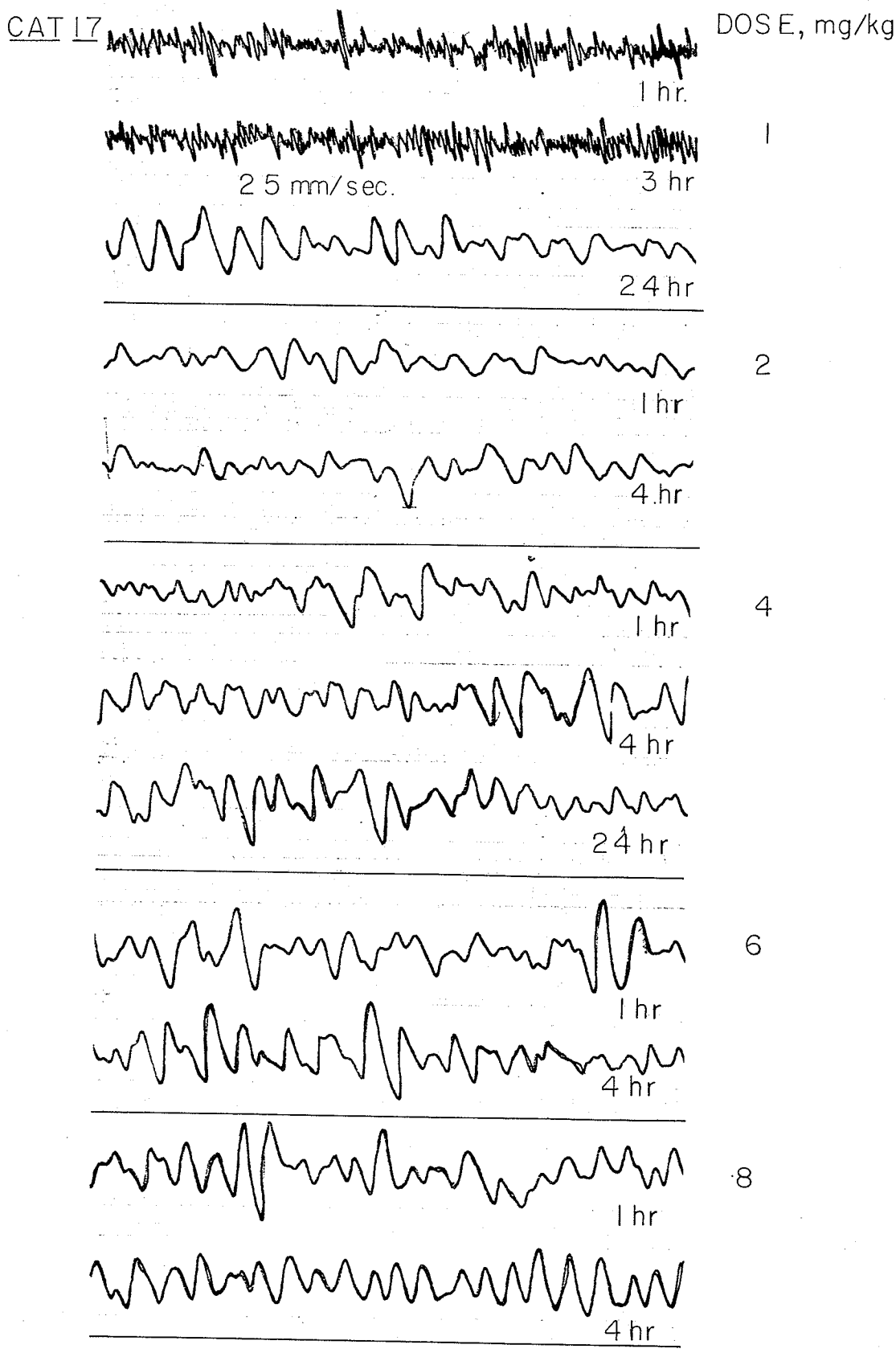
Appendix. Figure IV. Sample tracings of the ECoG patterns of the cats administered Librium^{R}. Times and dose levels correspond to the mean frequencies listed in tables 2 and 3.



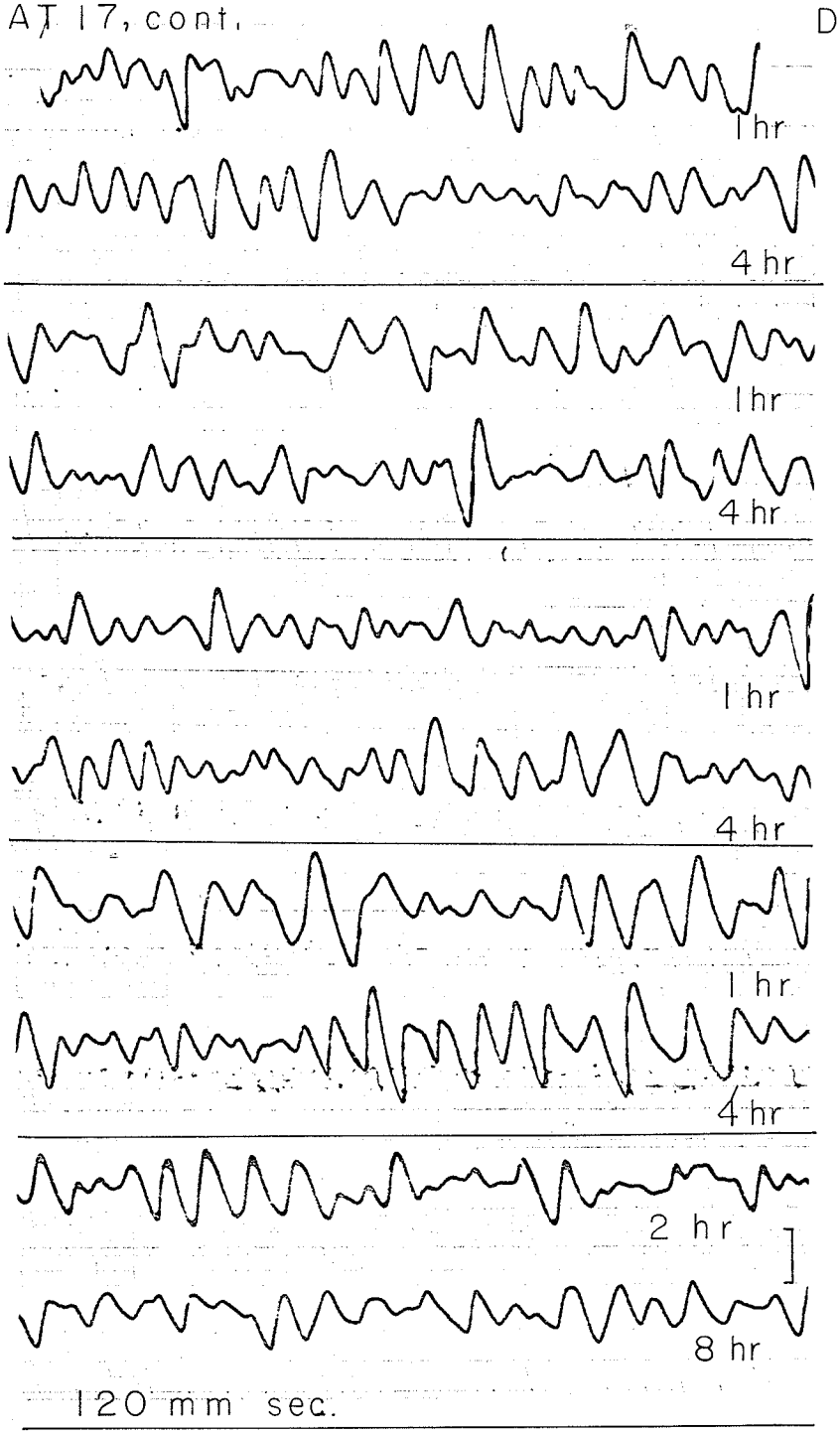
Appendix. Figure IVa. Sample ECoG tracings before injection.

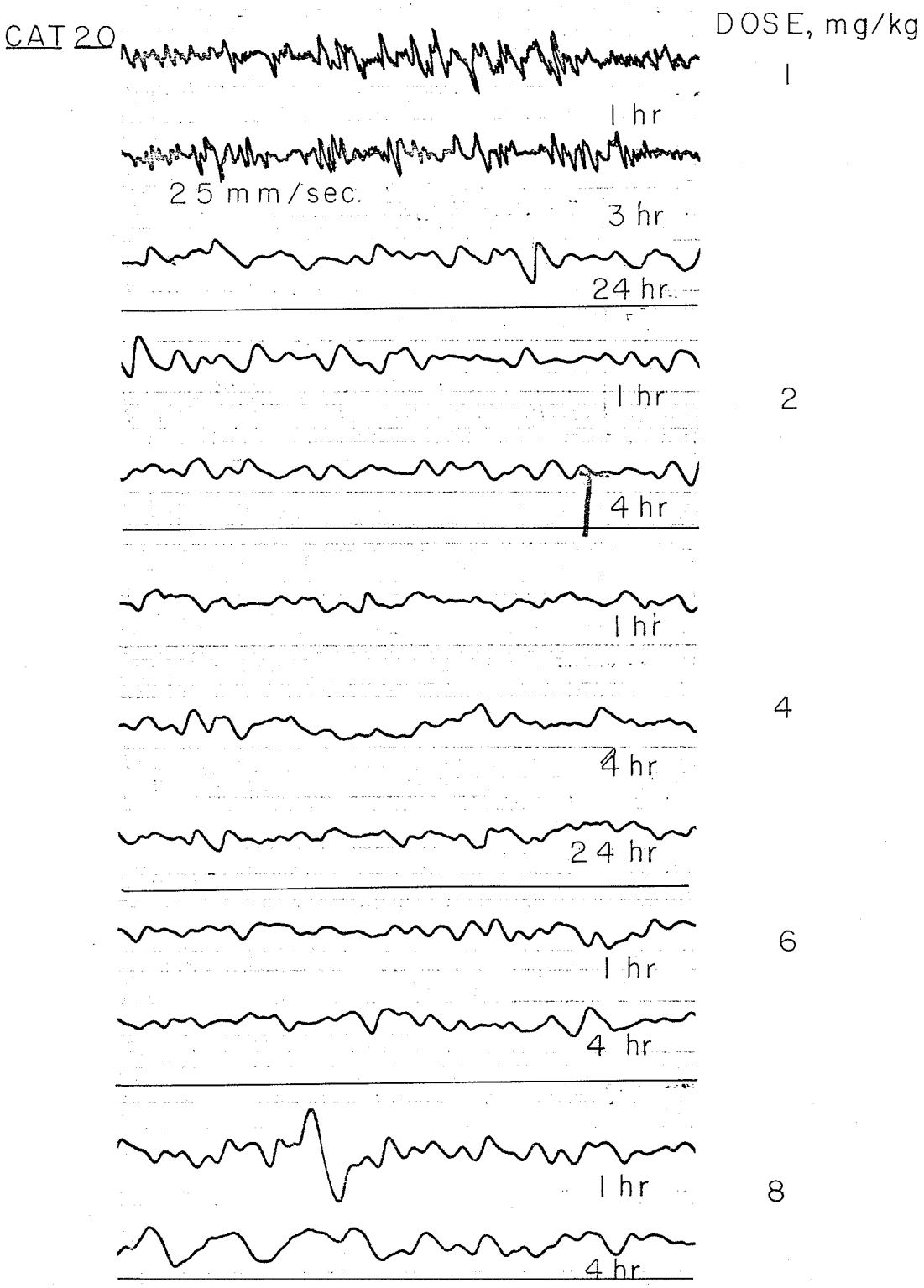


Appendix. Figure IVb. Sample ECoG tracings 2 hours after control injections. The first four are from saline-injected cats. The last two were given benzyl alcohol.

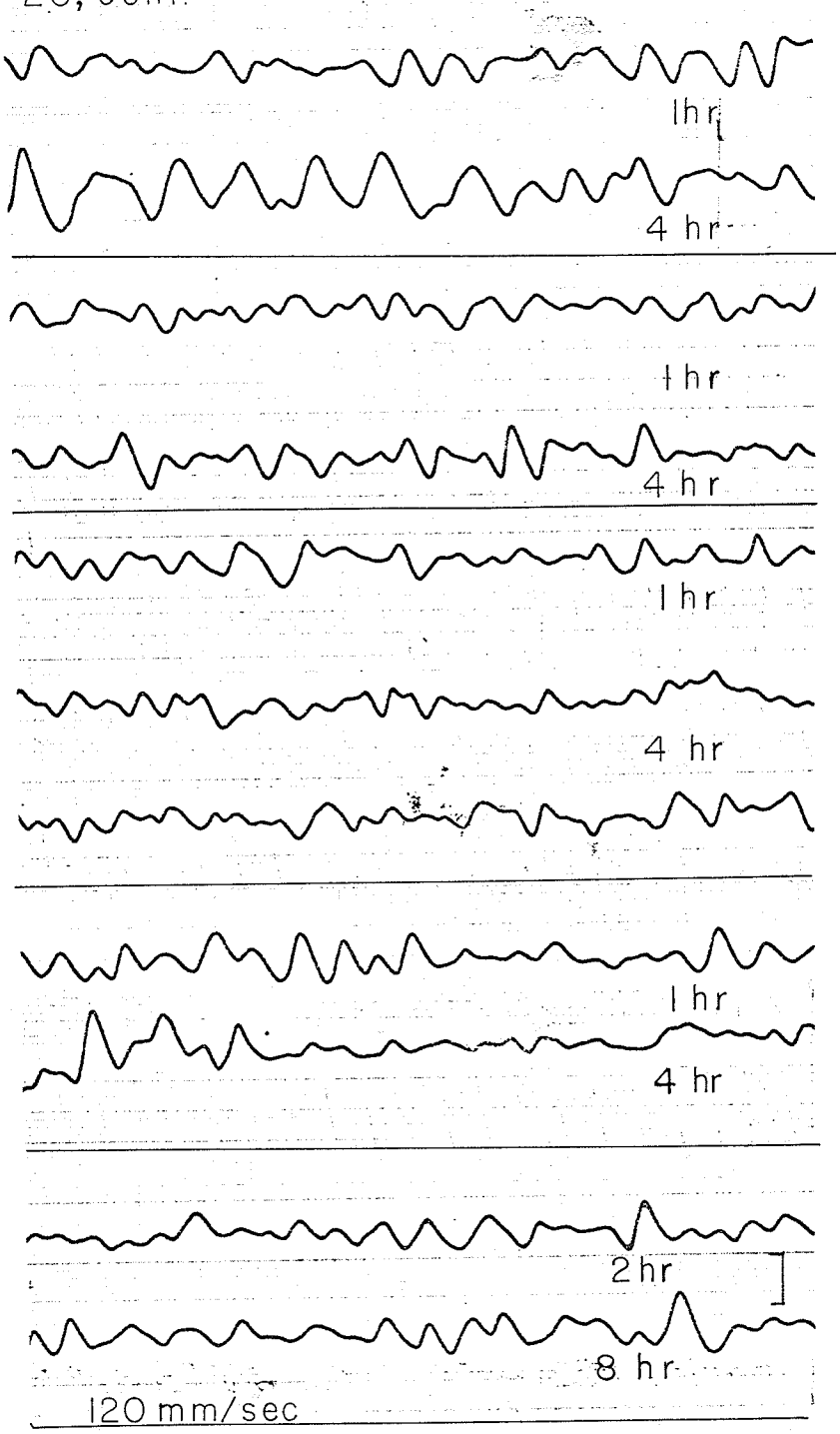

CAT 17
DOSE, mg/kg
1 hr.
1
25 mm/sec.
3 hr
24 hr
2
1 hr
4 hr
4
1 hr
4 hr
24 hr
6
1 hr
4 hr
8
1 hr
4 hr

CAT 17, cont.

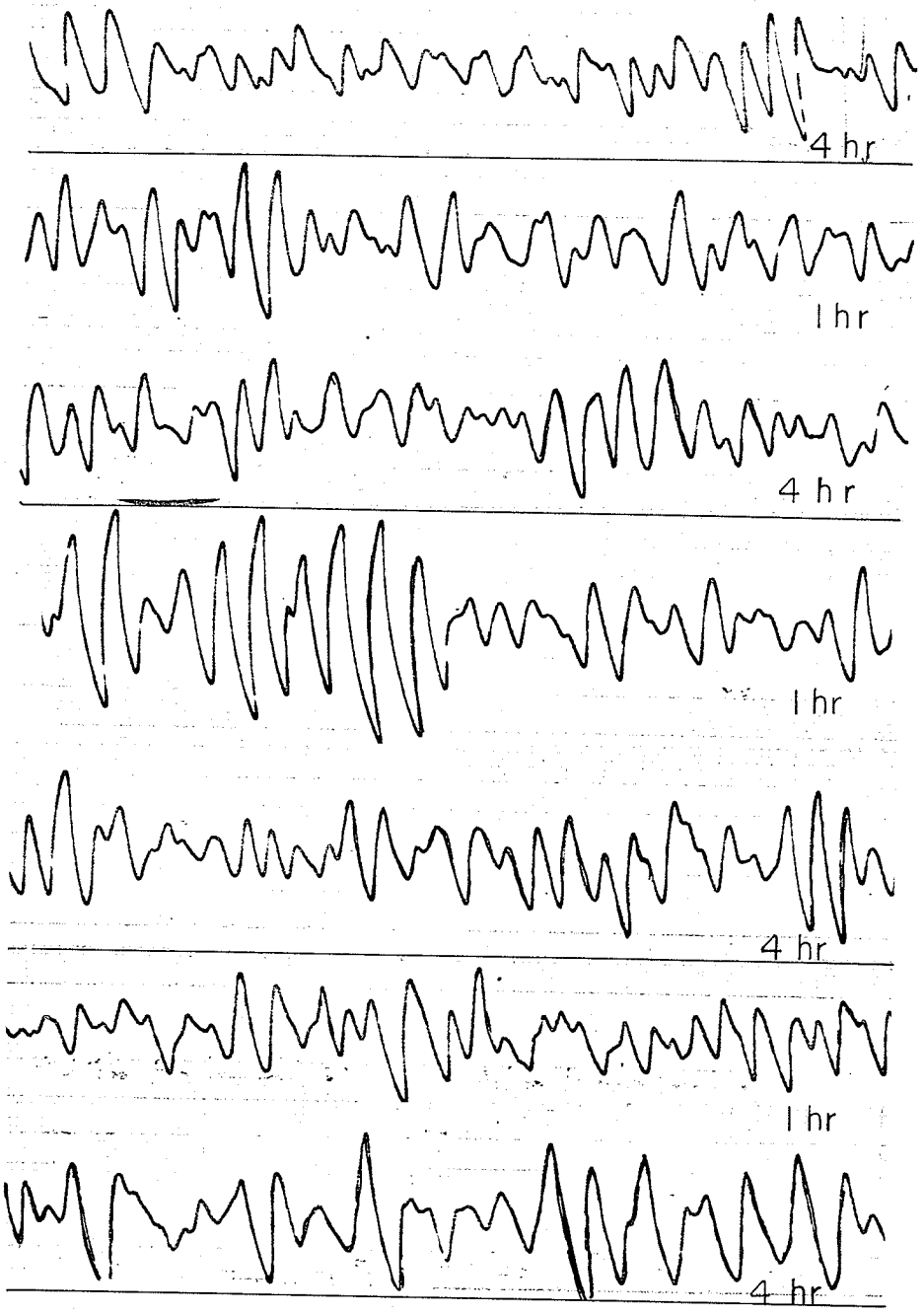

DOSE, mg/kg
1 hr
10
4 hr
1 hr
12
4 hr
1 hr
14
4 hr
1 hr
16
4 hr
2 hr
18
8 hr
120 mm sec.

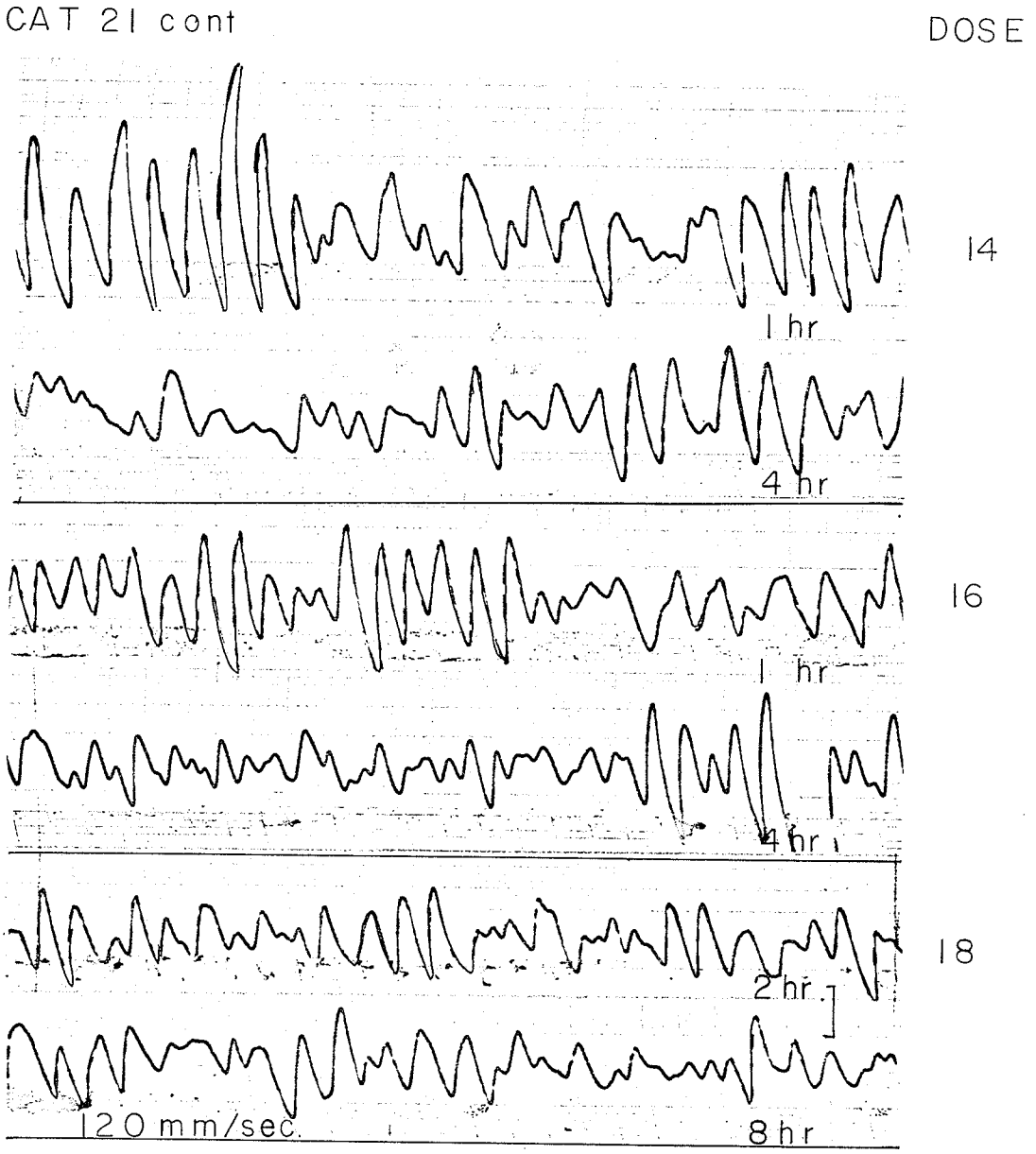

CAT 20
DOSE, mg/kg
1
1 hr
25 mm/sec.
3 hr
24 hr.
2
1 hr
4 hr
1 hr
4
4 hr
24 hr
6
1 hr
4 hr
1 hr
8
4 hr

CAT 20, cont.

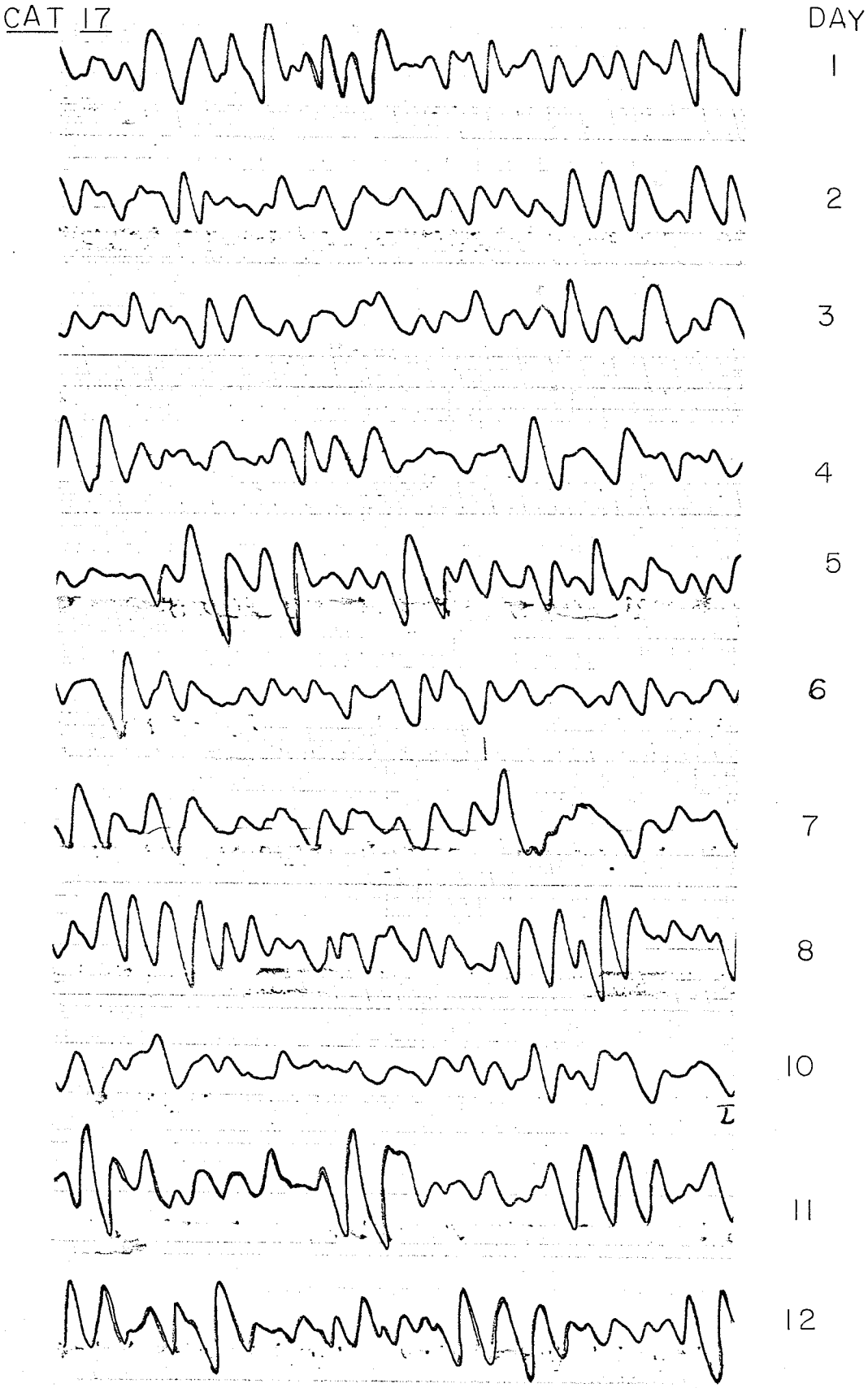

DOSE, mg/kg
10
1 hr
4 hr
12
1 hr
4 hr
14
1 hr
4 hr
16
1 hr
4 hr
18
2 hr
8 hr
120 mm/sec

CAT 21

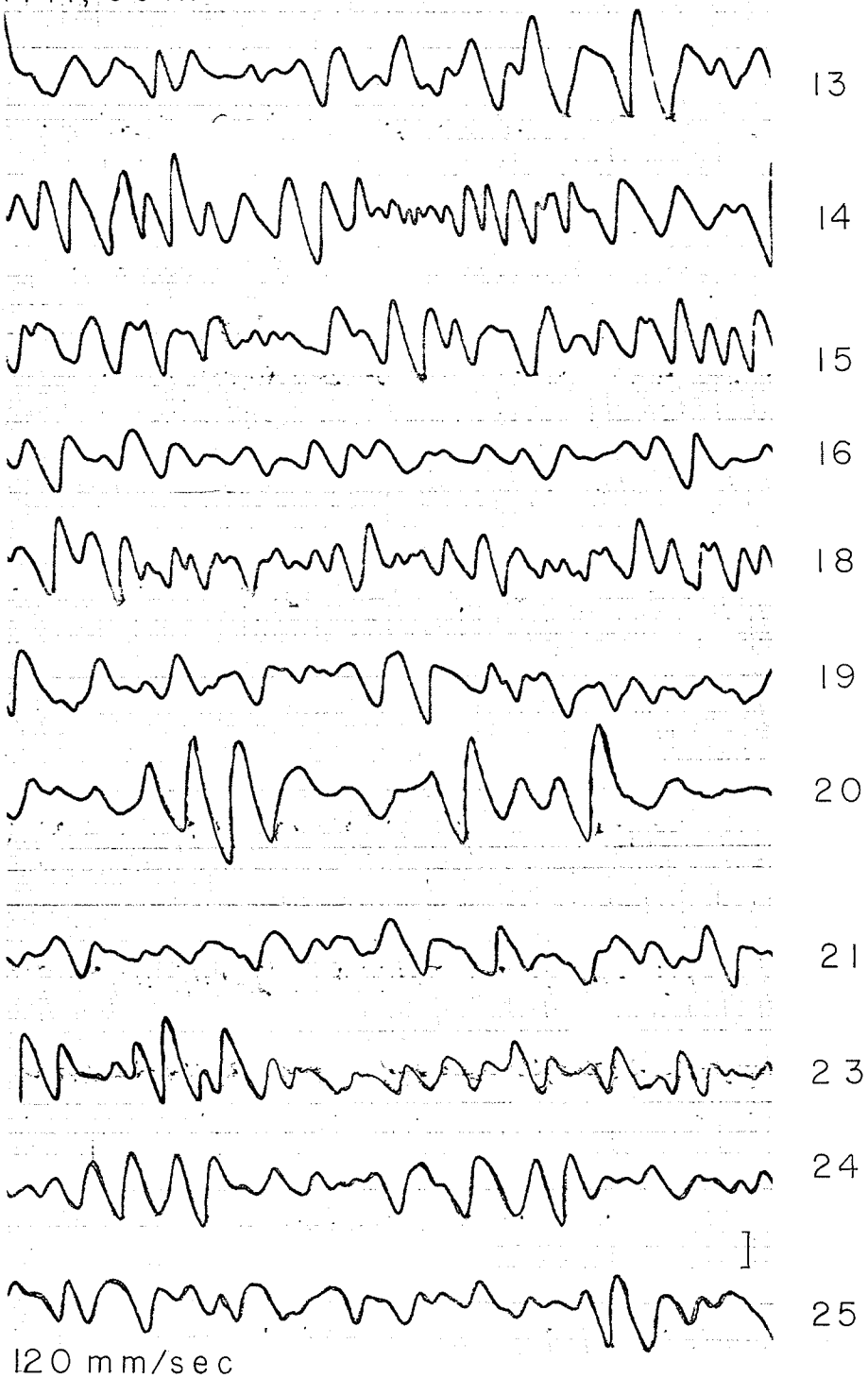


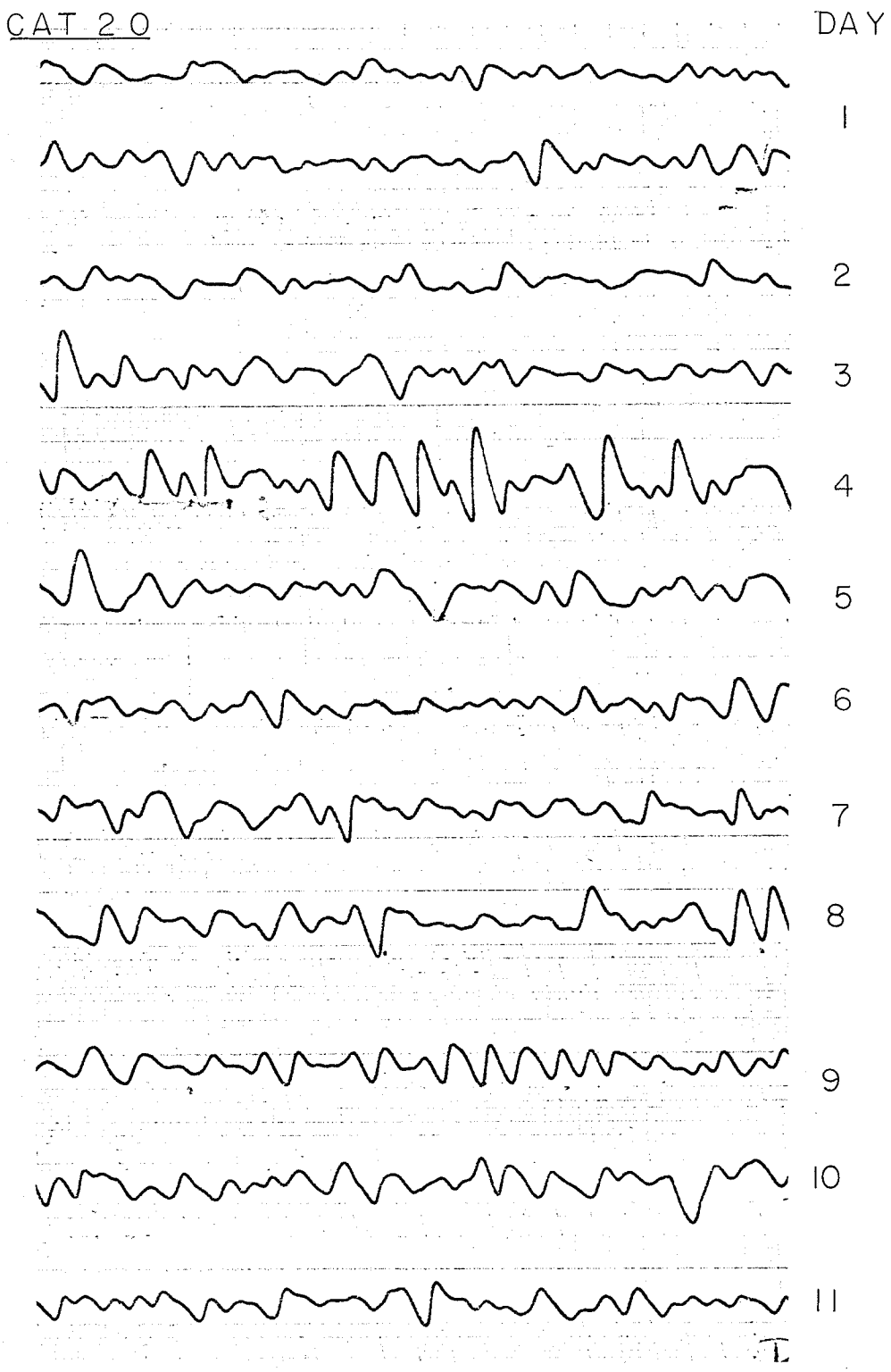


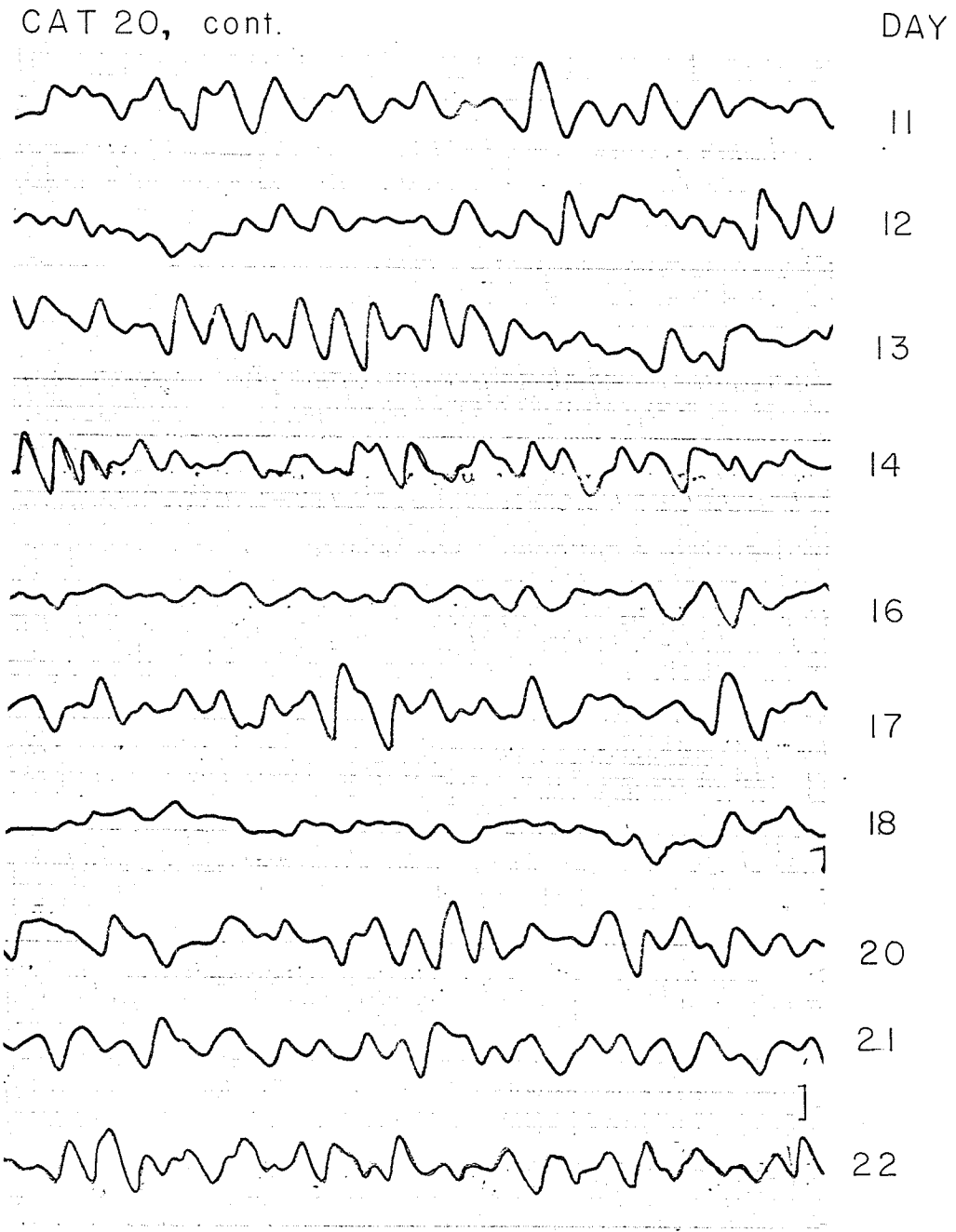
Appendix. Figure IVc. Sample ECoG tracings of cats 17, 20 and 21 at various hours post-injection of increasing doses of Librium®.


CAT 17
DAY
1
2
3
4
5
6
7
8
10
11
12

CAT 17, cont.

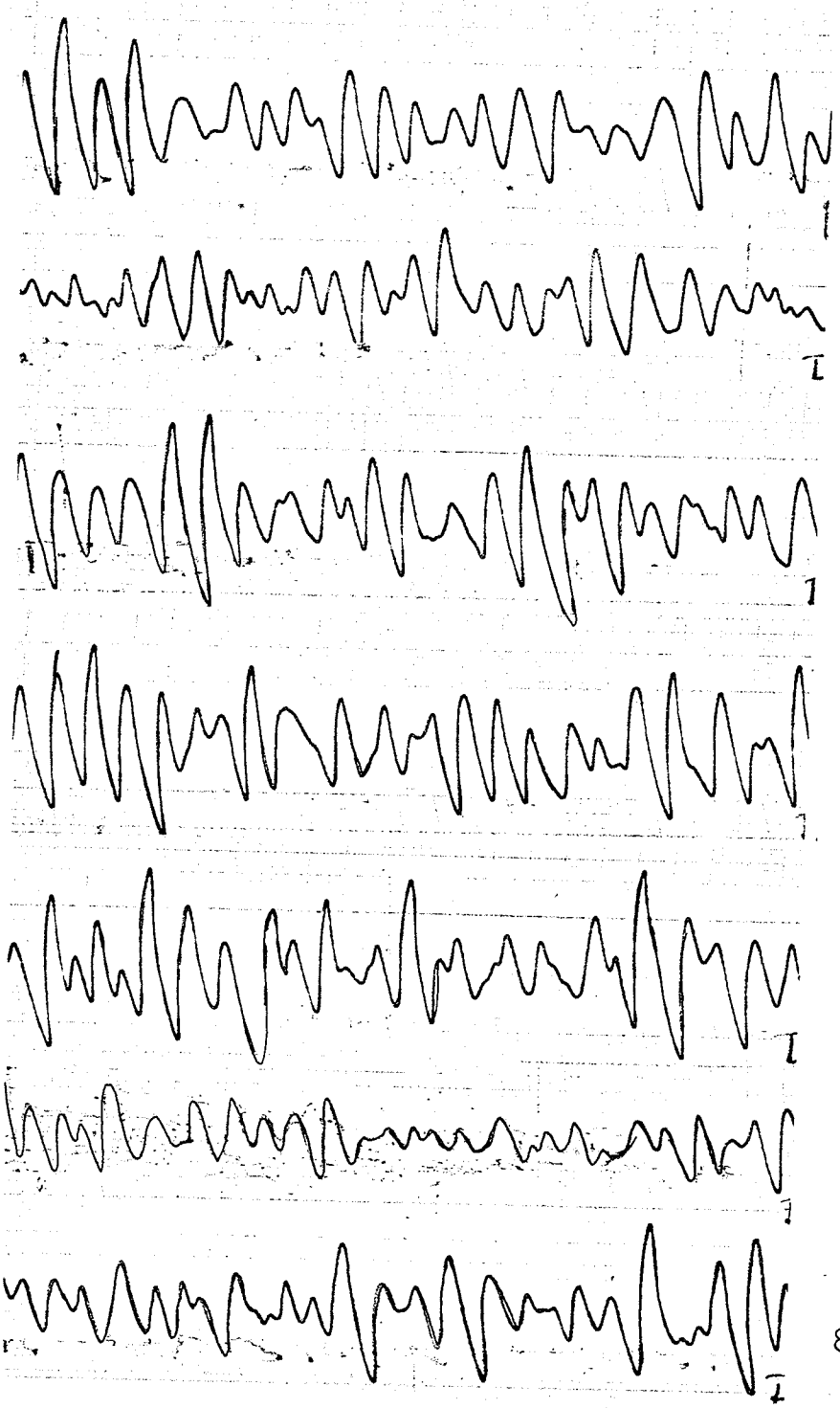



CAT 20
DAY
1
2
3
4
5
6
7
8
9
10
11

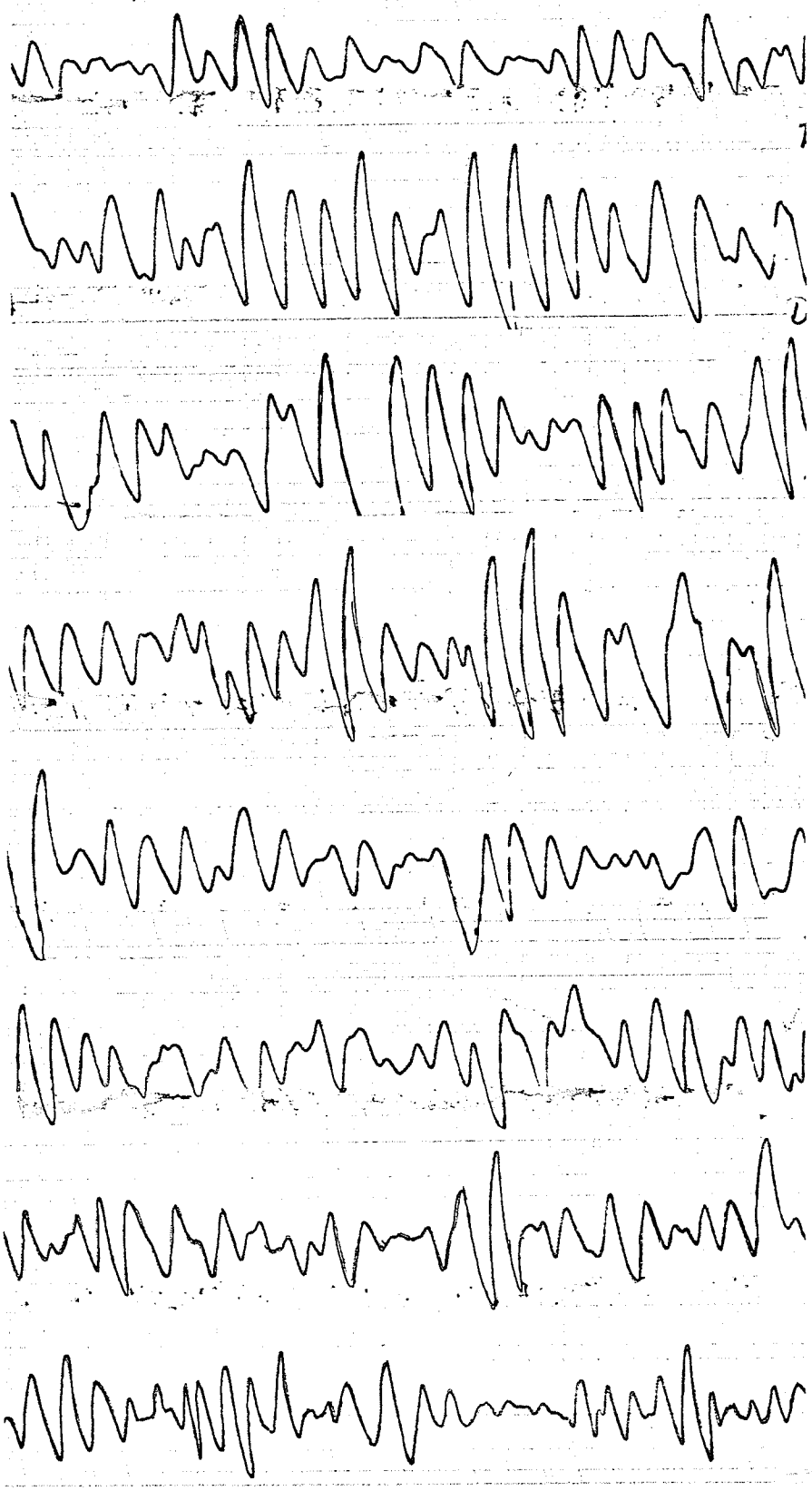

CAT 20, cont.
DAY
11
12
13
14
16
17
18
20
21
22
120 mm/sec

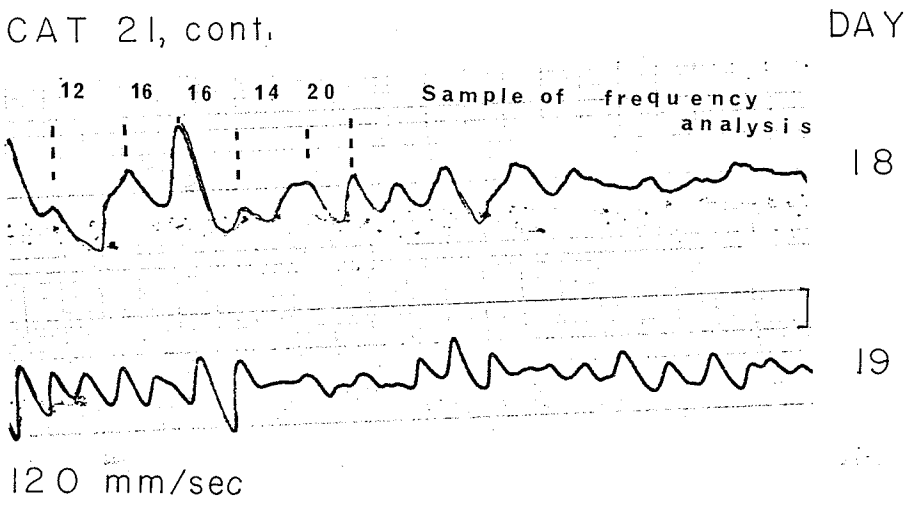
CAT 21

DAY



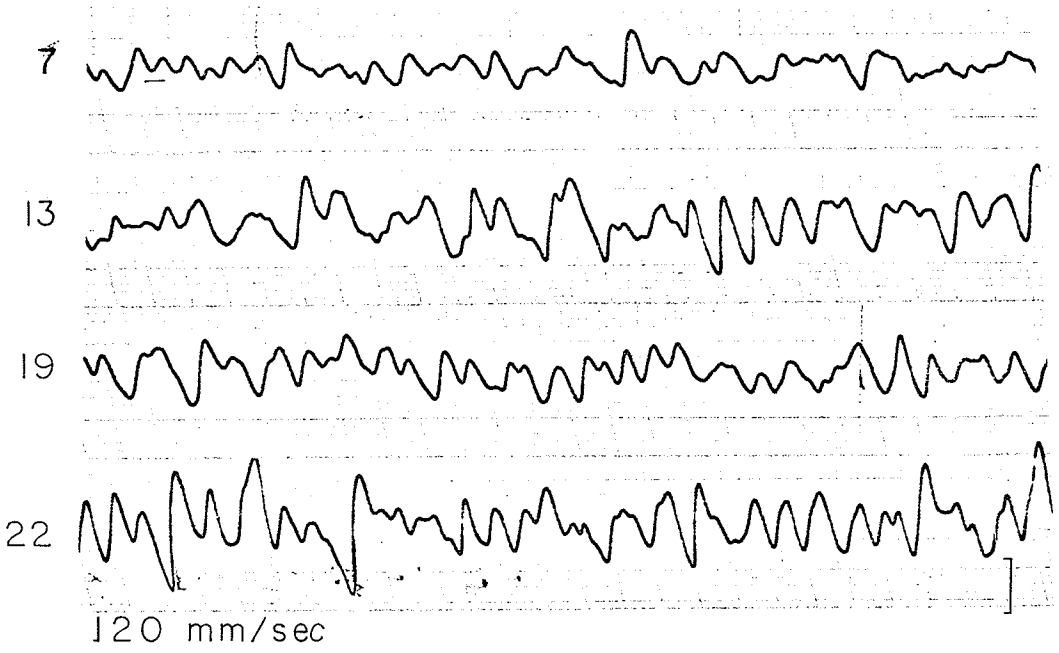
CAT 21, cont.



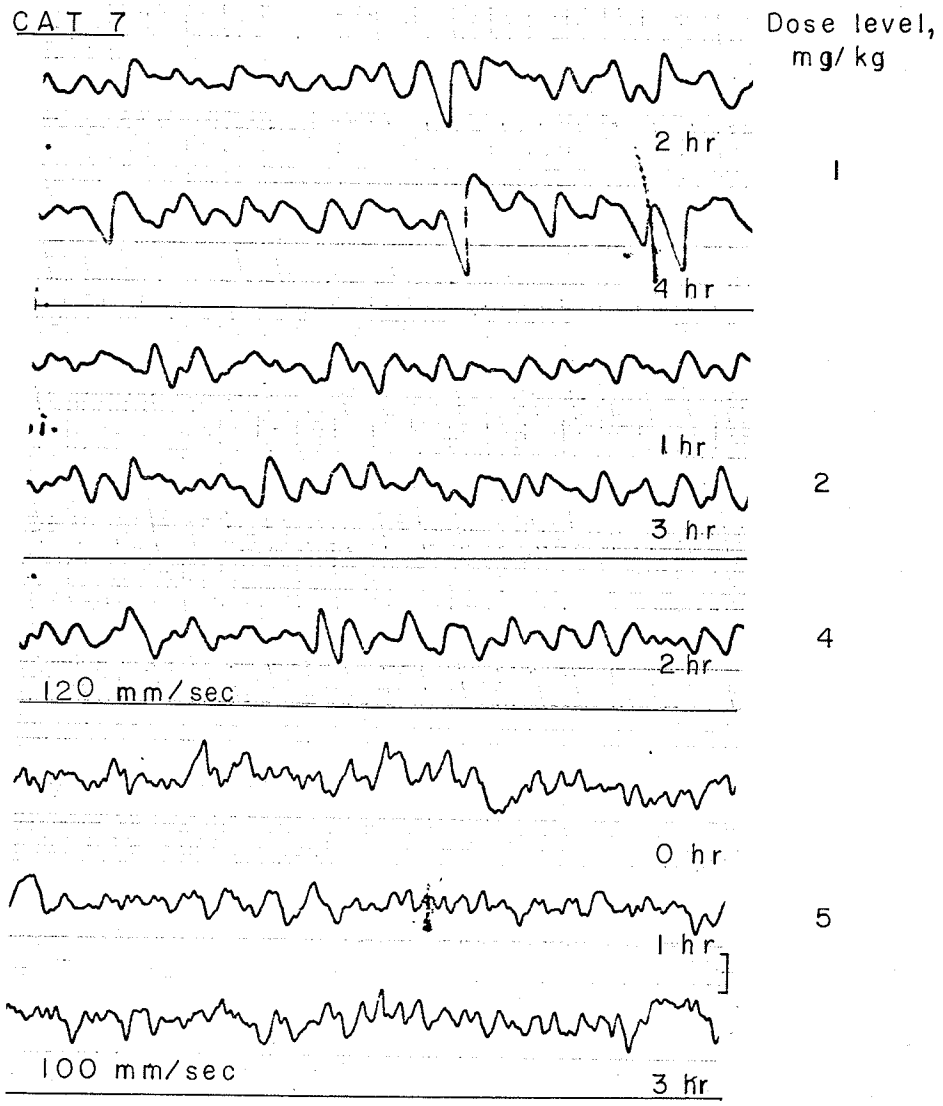


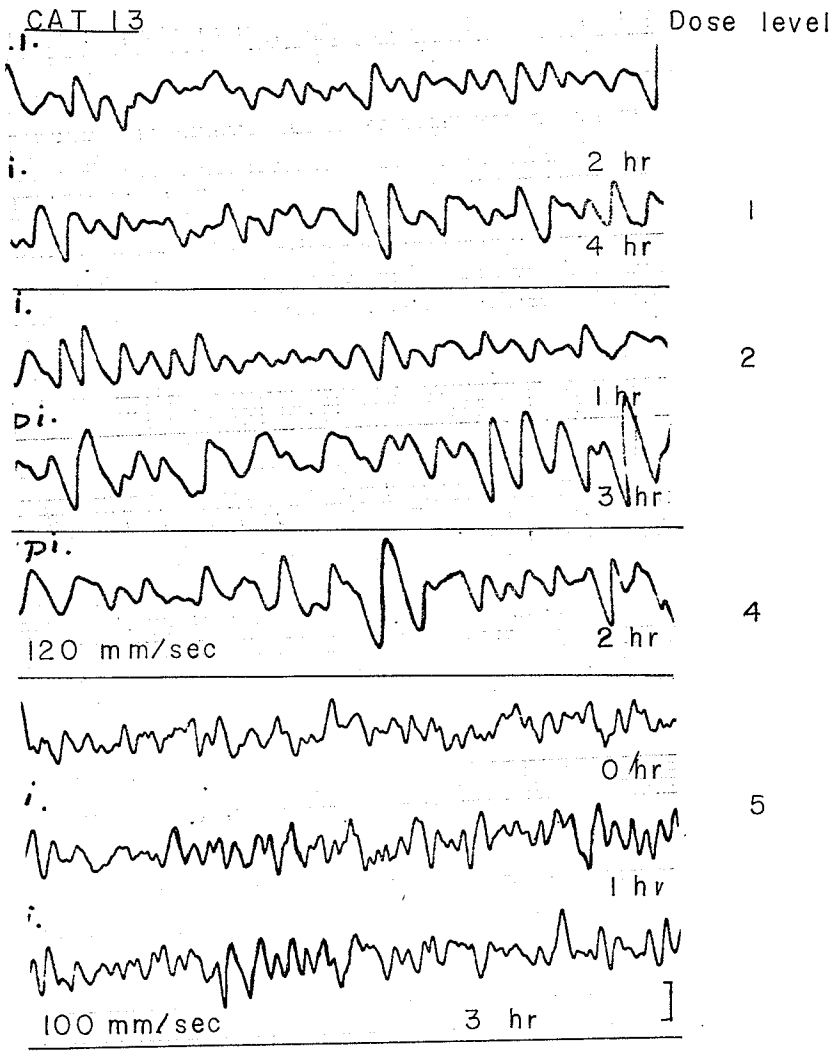
Appendix. Figure IVd. Sample ECoG tracings from cats 17, 20, 21 at daily intervals. Dose level was maintained at 16 mg/kg. b. wt. Librium^{R}.

Appendix. Figure V. Sample tracings of the ECoG patterns of the cats administered Valium^{R}. Times and dose levels correspond to the mean frequencies listed in tables 4 and 5.

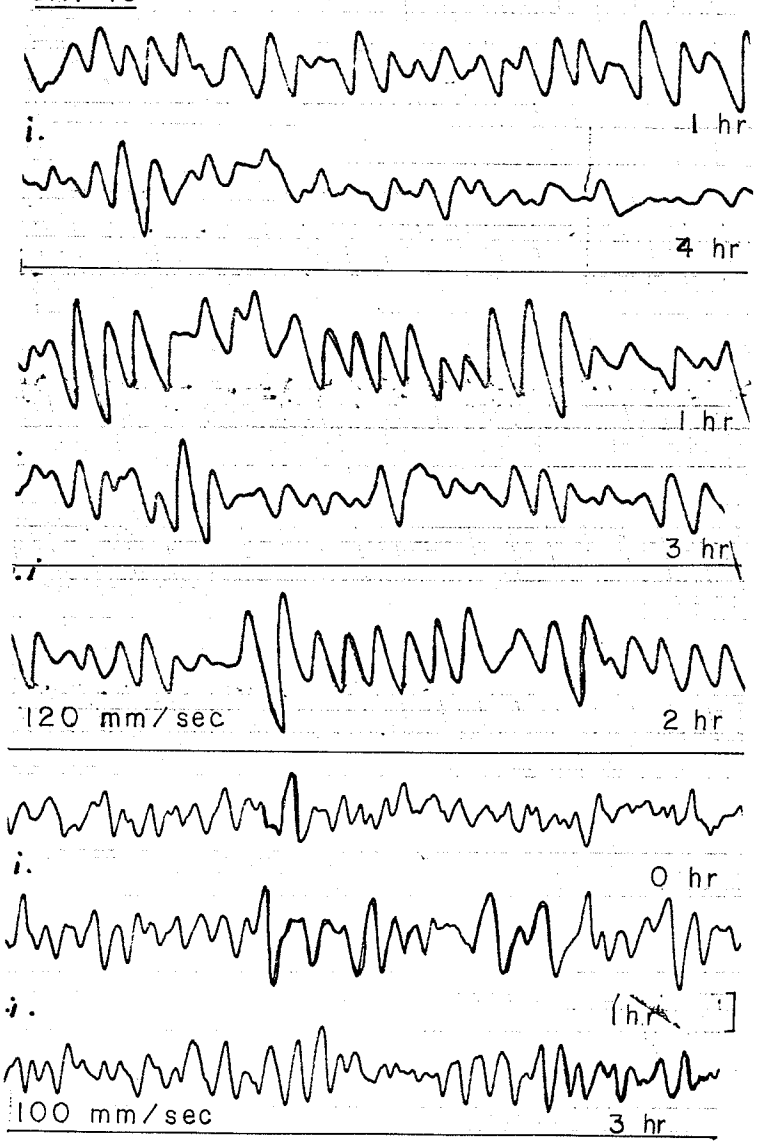


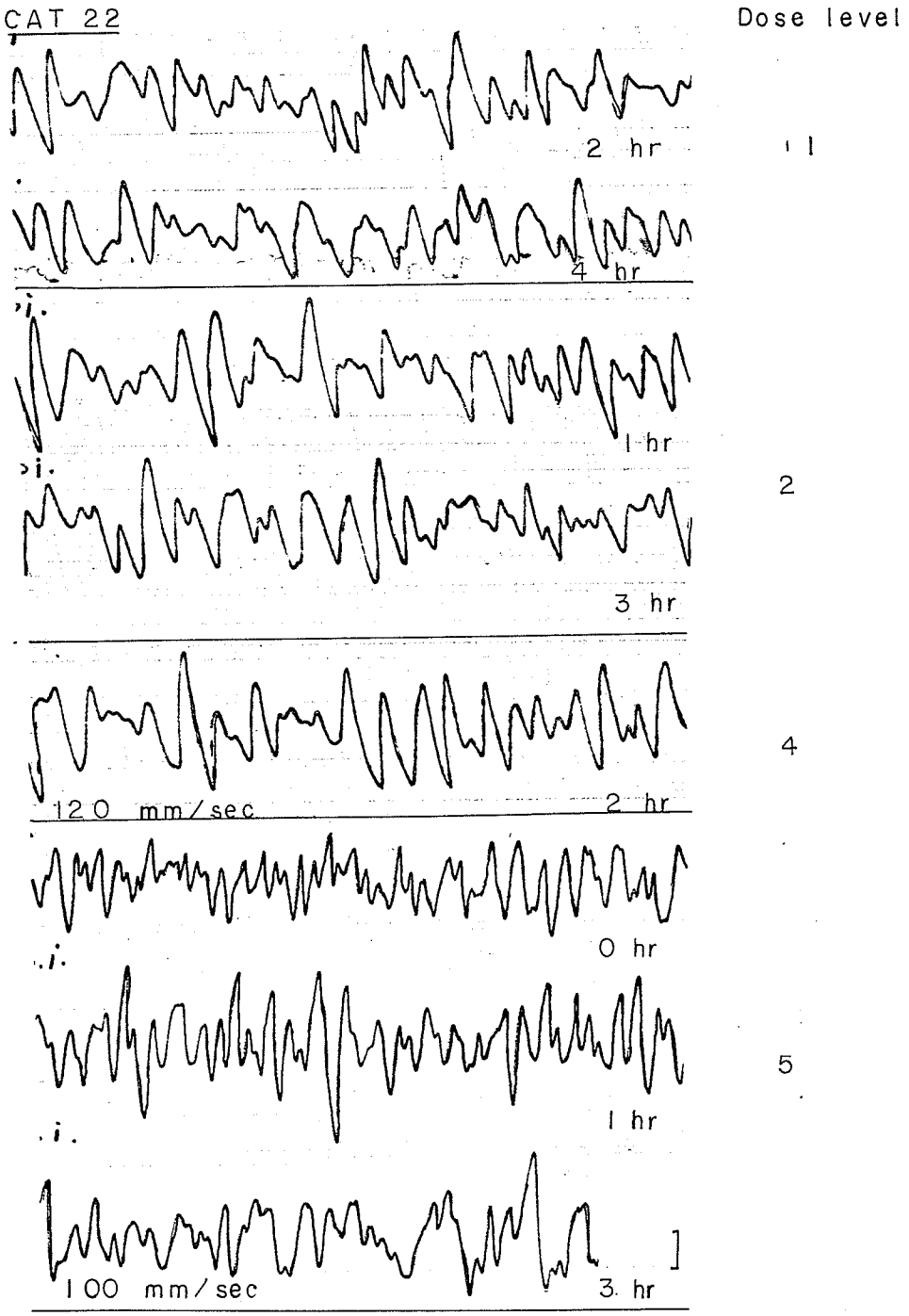
Appendix. Figure Va. Sample ECoG tracings before injection.


CAT 7
Dose level, mg/kg
2 hr
1
4 hr
1 hr
2
3 hr
4
2 hr
120 mm/sec
0 hr
5
1 hr
100 mm/sec
3 hr


CAT 13
Dose level
i.
2 hr
i.
4 hr
1
i.
2
1 hr
pi.
3 hr
pi.
4
120 mm/sec
2 hr
0 hr
i.
5
1 hr
i.
100 mm/sec
3 hr

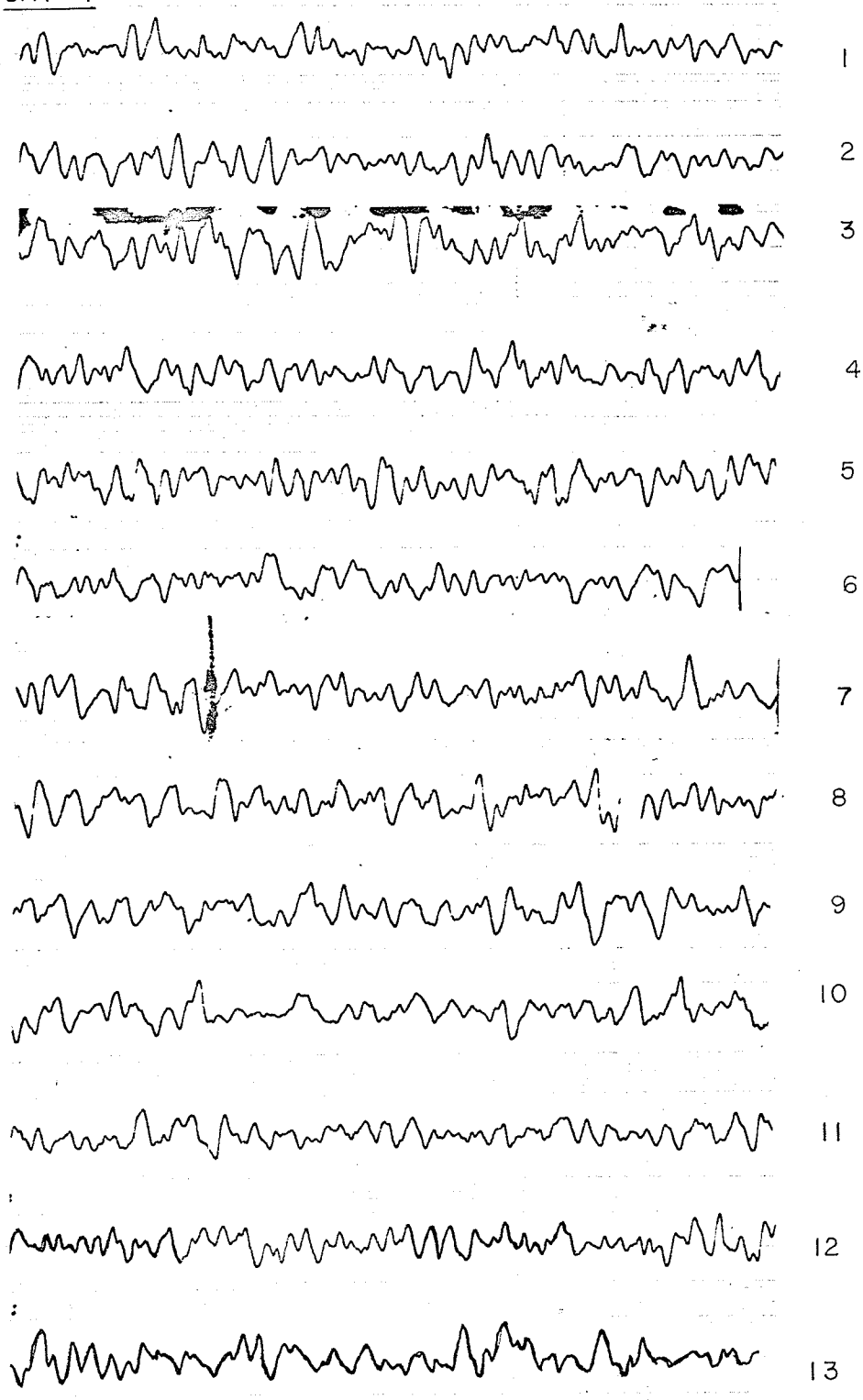
CAT 19


Dose level
1
1 hr
4 hr
1 hr
2
3 hr
4
120 mm/sec
2 hr
0 hr
5
3 hr
100 mm/sec
3 hr

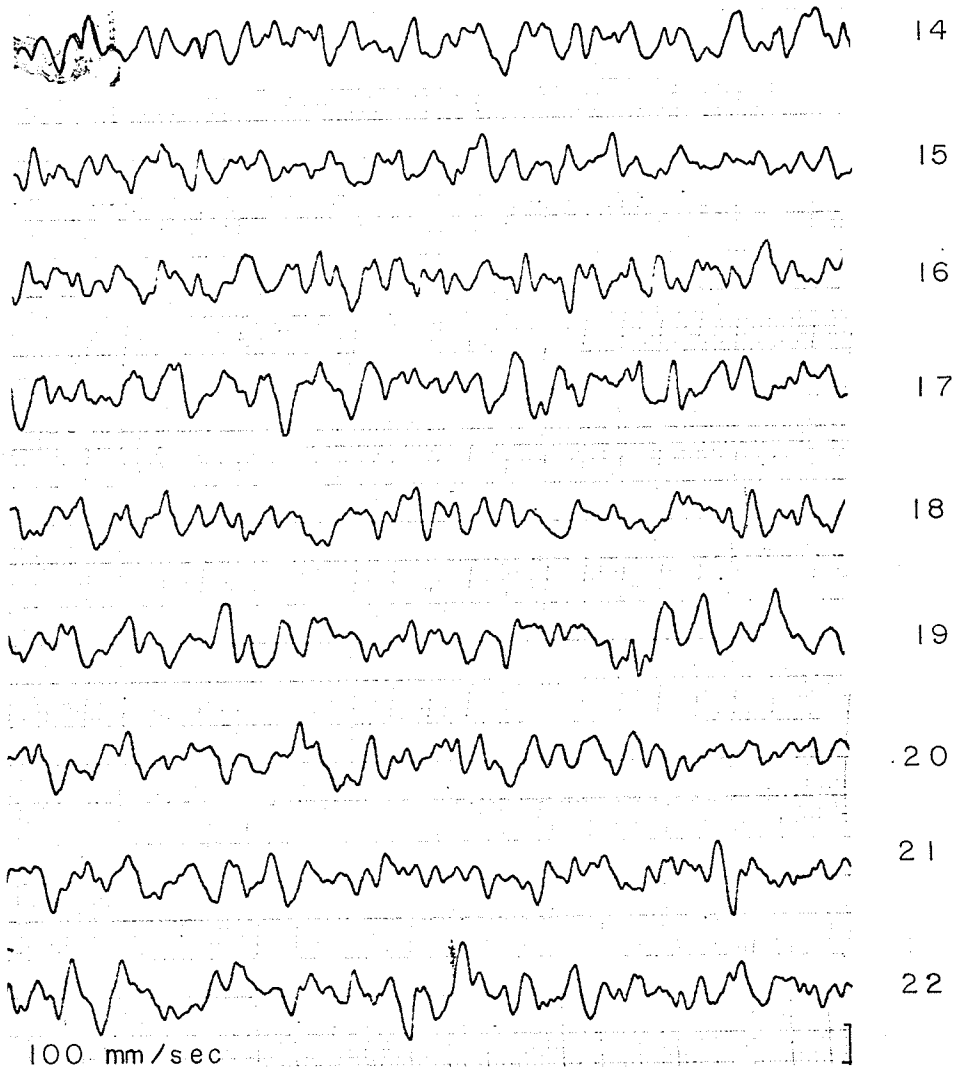


Appendix. Figure Vb. Sample ECoG tracings of cats 7, 13 19, 22 at various hours post-injection of increasing doses of Valium^{R}.

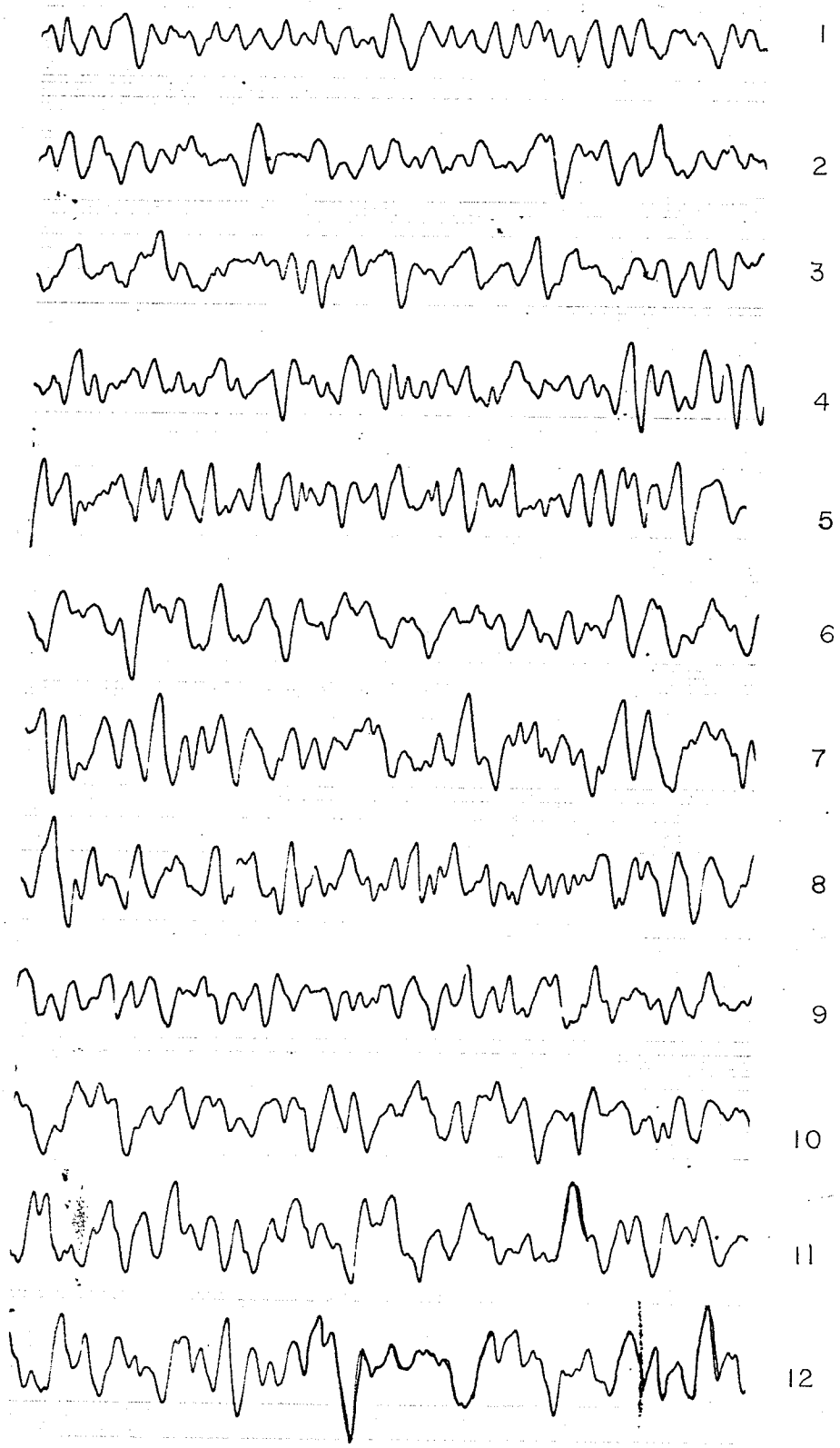
CAT 7



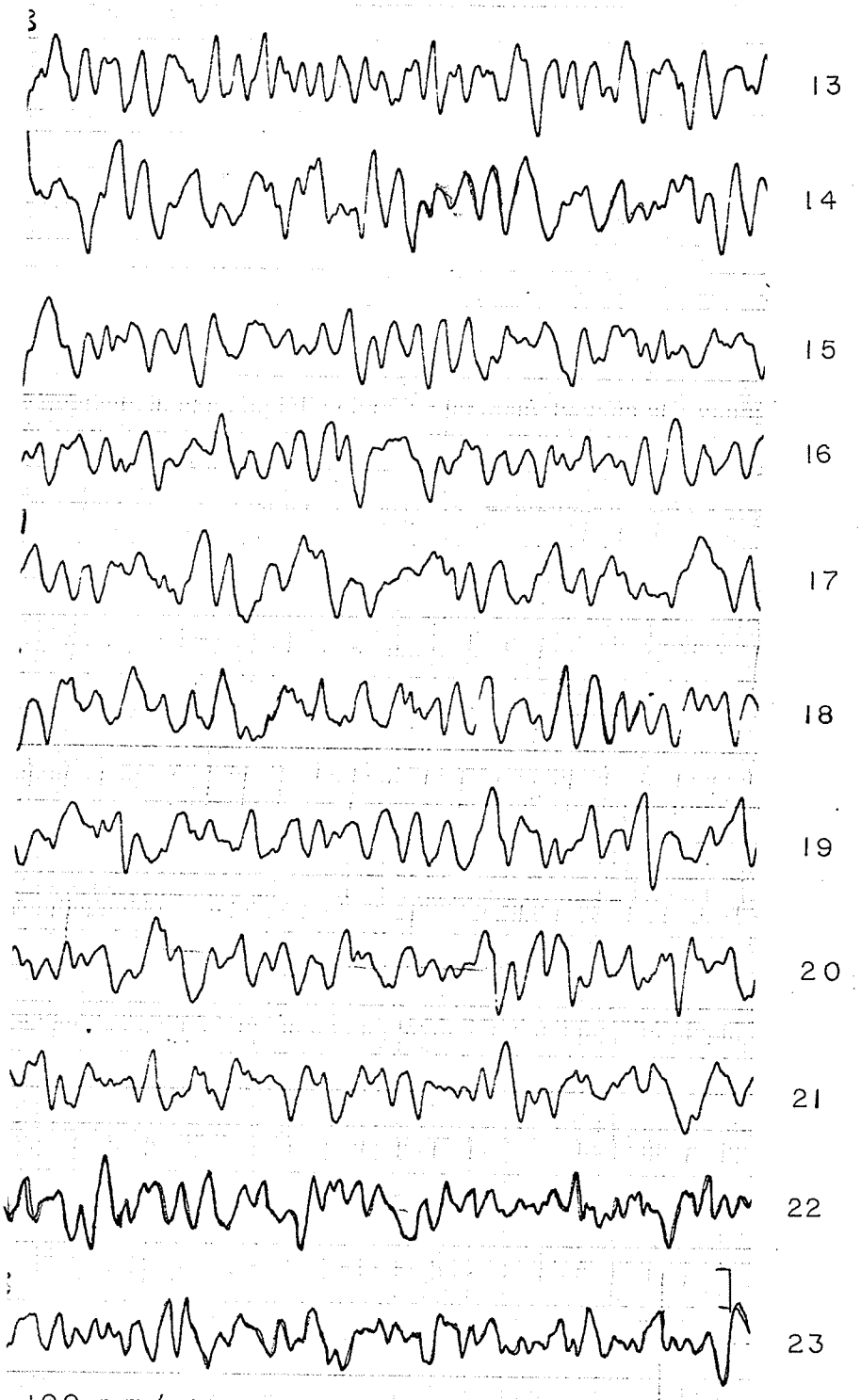
CAT 7, cont.



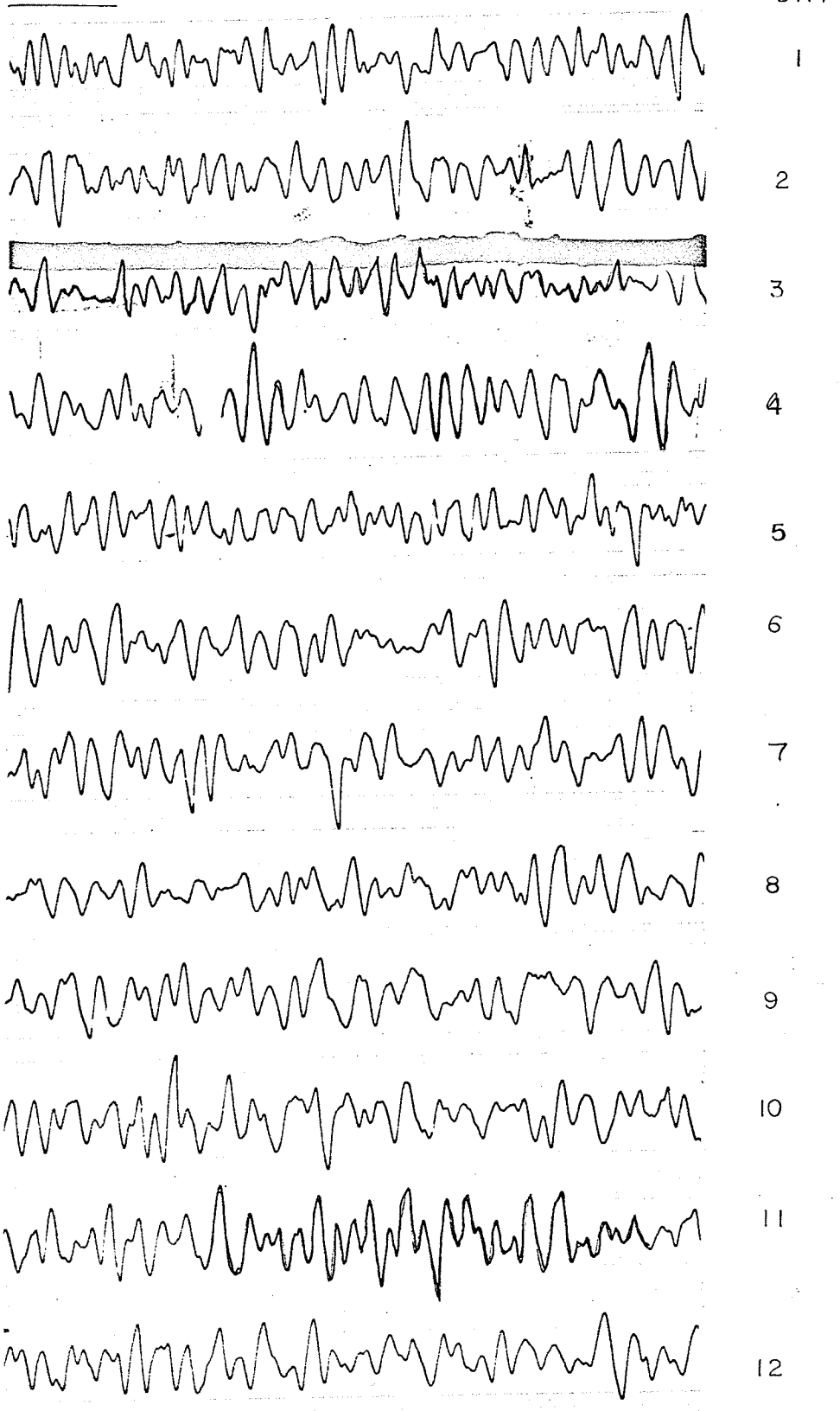
CAT 13



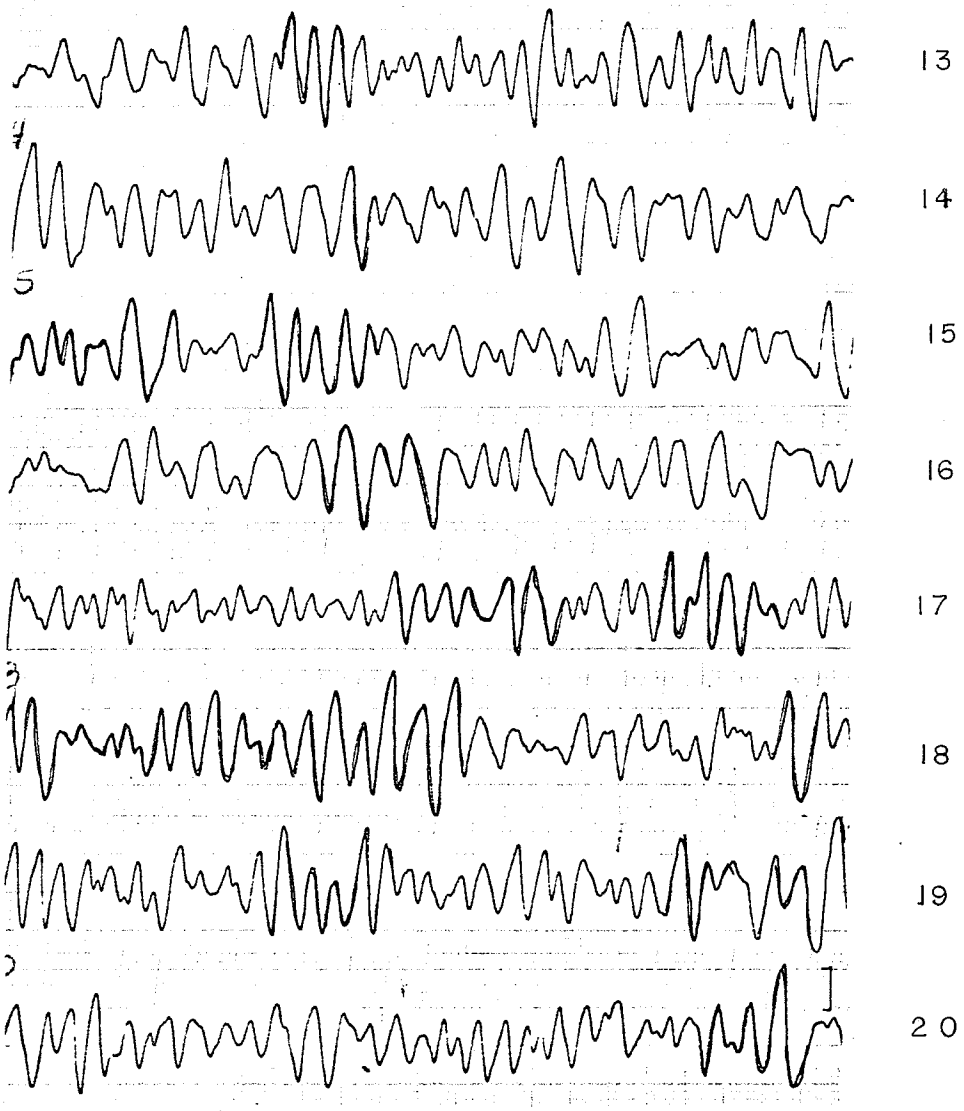
CAT 13, cont



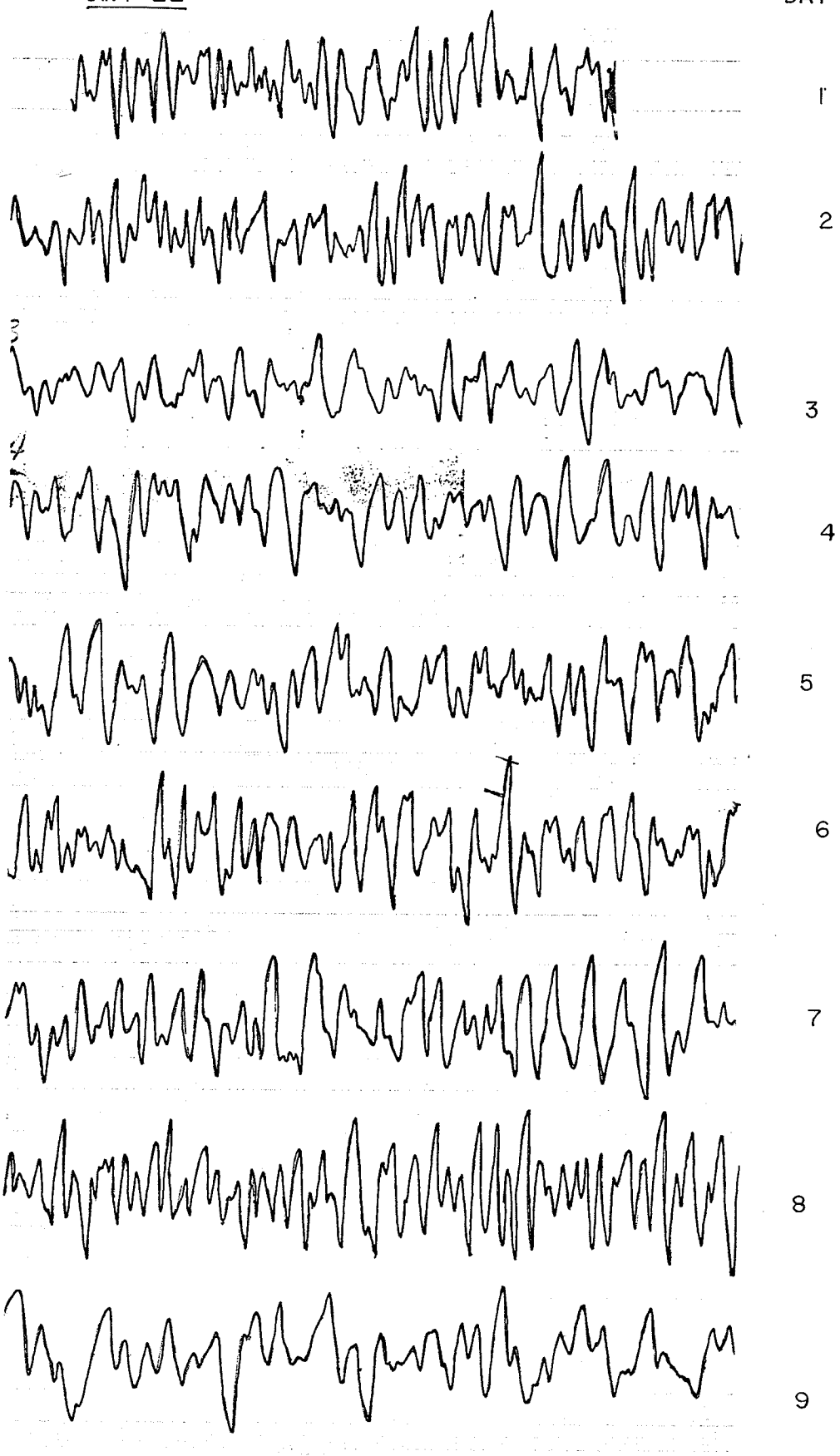
CAT 19



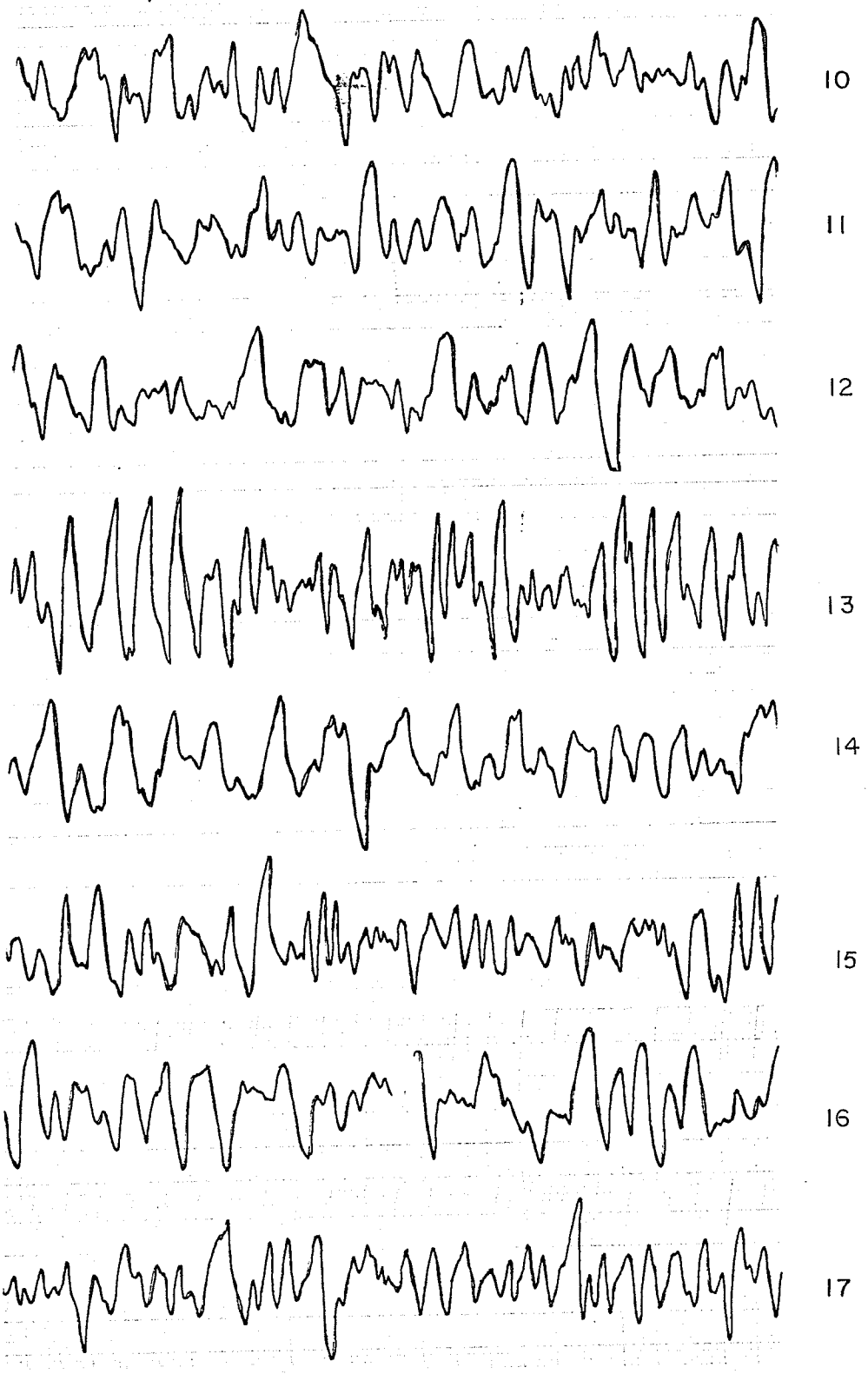
CAT 19, cont. ——

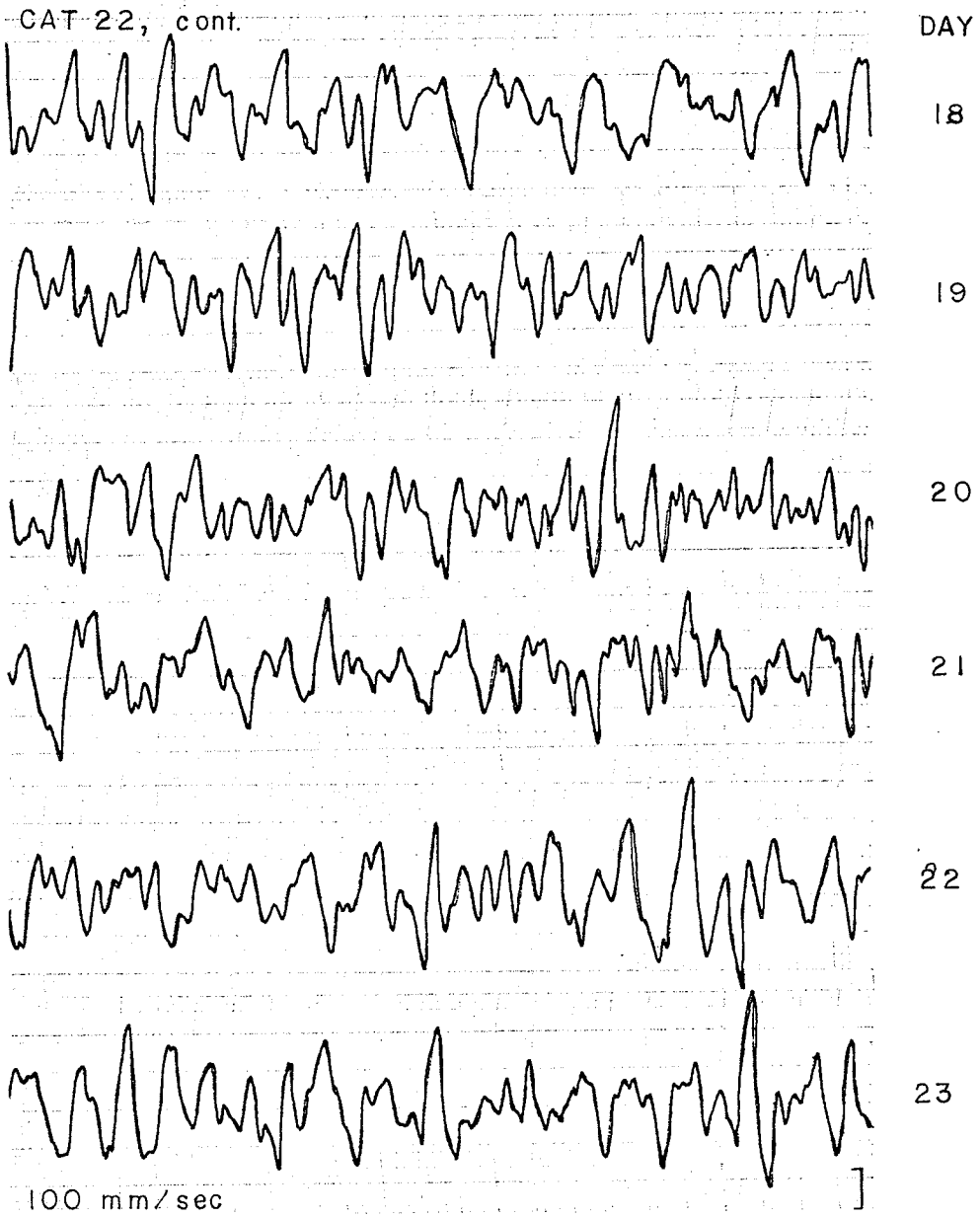


CAT 22



CAT 22, cont.





Appendix. Figure Vc. Sample ECoG tracings from cats 7, 13, 19, 22 at daily intervals. Dose level was maintained at 5 mg/kg. b. wt. Valium^{R}.