PATHOPHYSIOLOGY OF GENERALIZED PENICILLIN EPILEPSY IN THE CAT: THE ROLE OF CORTICAL AND SUBCORTICAL STRUCTURES

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PATHOPHYSIOLOGY OF GENERALIZED PENICILLIN EPILEPSY IN THE CAT: THE ROLE OF CORTICAL AND SUBCORTICAL STRUCTURES

by

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ABSTRACT

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Pathophysiology of generalized penicillin epilepsy in the cat: The role of cortical and subcortical structures.

Intramuscular injection of high doses (300,000 - 400,000 I.U./kg) of penicillin in the cat produces a transient epileptic state resembling human myoclonic petit mal in its electroencephalographic and clinical manifestations.

It was found that the generalized bilaterally synchronous epileptiform discharges in this condition are triggered with a high degree of probability by single shock or low frequency stimulation of structure (mostly the unspecific thalamic nuclei) from which before penicillin spindles or recruiting responses were elicited by such stimulations. Similar stimulations of other subcortical and cortical structures were much less effective.

Diffuse bilateral cortical application of a weak penicillin solution reproduced the electrographic syndrome of feline generalized penicillin epilepsy, including its characteristic response pattern to single shock and low frequency stimulation.

Thalamic application of penicillin did not reproduce the syndrome.

The distribution of penicillin within the brain with the various modes of application of the drug was measured by using radioactive C_{14} Penicillin.

RÉSUMÉ

Luis Felipe QUESNEY

Physiopathologie de l'épilepsie généralisée pénicillinique du chat

L'injection intramusculaire de hautes doses (300,00 à 400,000 U.I./kg) de pénicilline chez le chat produit un état épileptique transitoire qui ressemble au petit mal myoclonique de l'homme dans ses manifestations électroencephalographiques et cliniques.

Il a été établi que les décharges bilatérales synchrones épileptiques dans ce modèle d'épilepsie peuvent être précipitées avec un haut degré de probabilité par la stimulation électrique sous forme de chocs isolés ou à basse fréquence de structures (surtout les noyaux non spécifiques du thalamus) à partir desquelles avant l'administration de pénicilline il était possible d'induire des fuseaux ou des réponses recrutantes par de pareilles stimulations. Des stimulations similaires appliquées à d'autres structures souscorticales ou corticales étaient beaucoup moins efficaces.

En appliquant au cortex des deux hémisphères une solution de pénicilline à faible concentration, il est possible de reproduire le syndrome électrographique de l'épilepsie généralisée pénicillinique du chat. Les réponses caractéristiques de ce syndrome à des stimulations par chocs isolés ou à basse fréquence sont les mêmes que celles observées après injection intramusculaire de la drogue.

L'application de pénicilline au thalamus n'a pas reproduit le syndrome caractéristique de l'épilepsie généralisée pénicillinique du chat.

La distribution de la pénicilline dans le cerveau résultant des différentes modalités d'application de la drogue a été mesurée en utilisant de la pénicilline C_{14} radioactive.

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

I. The concept of petit-mal epilepsy (absence attacks): historical background.

"A knowledge of everything can be gained in two ways: through the definition of a thing and through its name, which (and it should be given only by wise and learned scholars) points out and depicts vividly the very nature and essence of the thing" (Galen, quoted by Lennox, Vol. 1, p. 42, 1960). In spite of this principle enunciated by Galen, the definition of a medical concept revealing its "very nature and essence" is in many instances a difficult task. In the field of epilepsy this difficulty is clearly made evident when one reviews ancient and even modern medical writings in which "wise and learned scholars" have attempted to define absence attacks and their probable mechanism.

Basically, petit mal seizures can be defined as a brief alteration in the state of consciousness occurring paroxysmally in transitory attacks without any warning. The patient is frequently unaware that an attack has occurred, but if he becomes aware of it, he may only notice that there has been a lapse in his stream of consciousness. The attacks may be accompanied by staring and muscular contractions involving facial muscles symmetrically.

Petit mal seizures have long been recognized as a characteristic clinical entity. In 1705 Poupart reported a clinical case to the Academie Royale des Sciences which undoubtedly exhibited some of the features of a petit mal seizure. During an attack the patient would sit down in a chair, open her eyes and remain immobile. "If she has begun to talk and the attack interrupts her, she takes it up again at precisely the point at which she stopped, and she believes she has talked continuously" (Temkin, 1945). In 1772 the Swiss physician Tissot reported a clinical case of a teenage girl suffering grand mal and petit mal seizures. "...in the intervals between the convulsions (grands accès") there were "petits accès" very frequent but very short almost of an instance duration, which were marked only by a momentary loss of consciousness, cutting short her speech, and by a slight trembling of the eyes. After regaining consciousness, she would finish her interrupted sentence but occasionally she had fogotten it" (Lennox, 1960). For the first time the word "petit" was used to describe a constellation of symptoms observed in an epileptic patient suffering petit mal seizures. The suffix "mal" was added to the word petit a few years later by Esquirol (1815) who reported: "..sometimes the attacks alternate in intensity: there are severe and slight attacks; this is what is called le grand and le petit mal in the hospitals" (Tempkin, 1945). However, Esquirol's definition of these two concepts was vague.

In spite of the lack of perfect definition, these terms have been widely accepted by medical practitioners and some of them are being employed even nowadays. Calmeil, Esquirol's pupil, in his doctoral thesis of 1824, introduced the concept of "absence" to denominate episodes of brief loss of consciousness occurring in epileptic patients (Tempkin, 1945; Gastaut, 1972). A few years later Delasiauve, in his "Traité de L'Epilepsie" (1854) finally assigned a distinctive place to the absence attacks among other cerebral seizures defining it as an abrupt loss of consciousness lasting a few seconds. (Gastaut, 1972; Lennox, 1960).

Up to this point, early neuroscientists and epileptologists were mainly interested in the proper definition, description and classification of the different types of epilepsies rather than concerned about the mechanisms involved in their genesis. Hughlings Jackson's observations and speculations on epilepsy in the second half of the nineteenth century, had a significant influence on the understanding of the pathophysiology of petit mal seizures. He considered that epileptic seizures were caused by "discharging lesions". "...epilepsy is a sudden excessive, and rapid discharge of grey matter of some part of the brain; it is a local discharge" (Jackson, Selected Writings, Vol. 1, p. 94). Although he made most of his observations on patients with focal seizures, he believed that this concept could be applied to all varieties of epilepsy. Keeping in line with this argument, he considered petit mal

epilepsy as being the result of a discharging lesion localized in the anatomical substrata of consciousness "the highest centres of the cerebral hemispheres" (Jackson, Vol. 1, p. 193). It is noteworthy that Jackson indicated a cortical mechanism of action in the genesis of the "highest level seizures" among which he included vertigo, petit mal and grand mal. "Highest level fits are those of the so-called idiopathic, or, as I may roughly say, they are ordinary epileptic fits; I suppose that most of these seizures depend on discharge - lesion of some part of the prefrontal lobe (motor province of the highest level) of one half of the brain". (Jackson, Vol. 2, p. 433).

II. The beginning of a new era.

The pioneer observations of Hans Berger (1929) leading to the discovery of electroencephalography, signalled the beginning of a new era in the clinical and neurophysiological approach to neurological diseases. In fact, according to Berger's seventh report (Hans Berger on the electroencephalogram of man, pp. 205-206), this author in 1930 recorded the EEG activity in a patient suffering numerous minor seizures and absence attacks. A few years after the discovery of electroencephalography, this technique became a very useful diagnostic tool (Jasper and Carmichael, 1935; Gibbs, Davis and Lennox, 1935).

The group headed by Gibbs was particularly interested in the understanding of epilepsy and they successfully applied the electroencephalographic technique in a search for the neurologic basis of this disorder. In 1935 these authors reported electroencephalograms obtained from 12 patients suffering characteristic petit mal attacks. In all these cases, the EEGs recorded during petit mal seizures showed outbursts of high voltage wave and spike activity at a frequency of 3/sec. They also noticed that the EEG pattern recorded in these patients between seizures was essentially normal. Shortly after Gibbs et al., reported this interesting finding, Penfield delivered the 1936 Harvey lecture (Penfield, 1938). This presentation was an event of utmost importance in the evolution of the pathophysiological concept of petit mal epilepsy. Based on his clinical observations on epileptic patients and using Hughlings Jackson's approach Penfield proposed at that time the foundation of what a few years later he would name the centrencephalic integrating system. Penfield was mainly concerned with the brain mechanisms subserving consciousness and therefore patients suffering absence attacks appeared to him as an excellent human model to study these mechanisms.

"On the other hand, loss of consciousness during a true seizure may be primary without any manifestation other than the blank expression and arrest of speech (petit mal)" ..."Consider for the moment that consciousness,

like movement, vision, hearing and speech, has a localizable representation in the brain"..."This topographic localization signified a belief not in a punctate center but in a general region".

"Consciousness is invariably lost at the beginning of the attack in which the convulsion is generalized from the start"..."Finally, there is much evidence of a level of integration within the central nervous system that is higher than that to be found in the cerebral cortex, evidence of a regional localization of the neuronal mechanism involved in this integration. I suggest that this region lies below the cerebral cortex and above the midbrain..."(Penfield, 1938).

The name "centrencephalic" was given to this integrating system in 1950 (Penfield, 1952, 1954). "I would propose the word centrencephalic to identify that system within diencephalon, mesencephalon and probably rhombencephalon which has bilateral functional connections with the cerebral hemispheres". "The centrencephalic system, then, might be defined as that central system within the brain stem which has been, or may be in the future, demonstrated as responsible for integration of the function of the two cerebral hemispheres".

The centrencephalic hypothesis was widely accepted because of the logical simplicity involved in such a concept. Since the epileptic discharge appears to begin all over the cortex at approximately the same time, it is logical to assume that some subcortical structures with

diffuse connections must be driving the cerebral cortex of the two hemispheres in a bilateral fashion.

In 1941, Jasper and Kershman and also Kornmueller proposed a subcortical diencephalic origin of the generalized and bilaterally synchronous spike and wave activity at 3/sec recorded in patients suffering petit mal attacks. This proposal, which conformed to Penfield's hypothesis, was mainly derived from electroencephalographic evidence obtained in a fairly large population of epileptic patients exhibiting generalized spike and wave activity in their EEGs.

The implication of the centrencephalic system as the site of origin of generalized seizures characterized by loss of consciousness from the start, stimulated neurophysiological research interest in the role of subcortical structures in relation to consciousness and mechanisms of EEG synchronization and desynchronization. Lewy and Gammon (1940) were the first to describe that thalamic stimulation in cats could induce a "cusp and dart" pattern recorded from the ectosylvian gyrus, which from an electrographic viewpoint resembled the wave and spike pattern reported by Gibbs et al., in patients with petit mal attacks.

It is noteworthy that the concept of a subcortical onset of generalized seizures was considered prior to the elegant neurophysiological studies of Morison and Dempsey (1942) which demonstrated the importance of a

restricted subcortical region (the midline intralaminar thalamic nuclei) for the elicitation of a generalized electrical response recorded over widespread areas of both cerebral hemispheres, the so-called recruiting response. The following step in the understanding of the pathophysiological role of subcortical structures in the regulation of the EEG was achieved when Moruzzi and Magoun (1949) described the desynchronizing effect exerted upon the EEG by stimulation of the mesencephalic reticular formation in cats. Further support for a subcortical origin of the generalized spike and wave activity in patients with petit mal epilepsy came from Hursch's (1945) observation that section of the corpus callosum in some patients did not disrupt the bilateral synchrony of the epileptic discharge. It should be pointed out though, that the validity of this observation is open to doubt because the section of the corpus callosum in these patients was incomplete.

Undoubtedly one of the most significant contributions to the centrencephalic hypothesis came from Jasper and Droogleever-Fortuyn's observations (1947) that low frequency stimulation of the intralaminar thalamic nuclei in anaesthetized cats could induce generalized and bilaterally synchronous cortical spike and wave activity with bifrontal predominance. The pattern of these discharges was similar to that observed in human cases of petit mal epilepsy. However, this response was inconstant, often unilateral, did not significantly outlast the end of the

stimulation delivered to the thalamus and the authors were unable to determine the exact experimental conditions necessary for its reliable production. Jasper's hypothesis of a thalamic pacemaker mechanism in centrencephalic epilepsy was supported by Hunter et al's (1949) findings, who demonstrated that thalamic stimulation in unanaesthetized cats could induce responses such as arrest of activity, staring and eye blinking, all of them resembling characteristic clinical features of petit mal attacks. Kopeloff et al. (1950) added further support to the centrencephalic hypothesis when demonstrating that topical injection of alumina cream into the medial nuclear groups of the thalamus in the rhesus monkey could induce recurrent convulsive seizures. This experimental result should be carefully interpreted because alumina cream could escape from the injection site through the needle tract, thus contaminating the cerebral ventricles, subarachnoid spaces or even the cortical surface, therefore exerting an epileptogenic action at various levels of the central nervous system. However, several authors including Jasper himself (Jasper and Droogleever-Fortuyn, 1947; Cohn, 1954, and Ogden et al., 1956), reported a lack of perfect synchronization of the spike discharge in spike and wave complexes recorded from homologous brain regions in patients with absence attacks. This consideration plus the knowledge that the massa intermedia is absent in about 30% of human

beings (Ogden et al, 1956) led to the proposal of a pacemaker mechanism located not in the midline thalamic nuclei as originally proposed by Jasper, but rather in thalamic structures aside from the midline bilaterally (Cohn, 1954) or in lower subcortical structures on either side (Ogden et al., 1956).

The results of animal experimentation favoring a subcortical onset of the electroclinical features of petit mal elipepsy stimulated interest in the study of the role of these structures in patients with petit mal. These studies were possible thanks to the development of stereotaxic surgical techniques which allowed the placement of electrodes into different subcortical structures in human beings. Although this approach appeared an adequate method to study the pathophysiology of generalized spike and wave activity in man, it was not exempt from serious technical limitations. Perhaps the most important criticism of these studies stems from 'patient selection'. This is so because patients suffering typical absence attacks usually do not require neurosurgical exploration. As a matter of fact, most of the cases reported in the literature suffered absence attacks in addition to various other seizures manifestations. Secondly, in the majority of the cases reported in the literature, the number of electrodes implanted in subcortical structures was too small providing only for sampling of the electrical activity

recorded from subcortical sites which was too limited to permit any serious conclusion regarding a possible onset of the epileptic activity in these structures. Furthermore, the position of the electrode tip was determined by radiological means, a technique which may cause some error in localization.

Spiegel (1951) reported evidence of a diencephalic mechanism involved in the genesis of spike and wave activity in patients with petit mal and grand mal seizures. The spike and wave activity in these cases was recorded simultaneously from the cortex and the thalamus, but the thalamic discharges outlasted the cortical ones. Furthermore, in a few cases in which EEG recordings were carried out under general anaesthesia, the epileptic discharges remained confined to the thalamus. Similar findings pointing towards a thalamic origin of the epileptic discharges seen in petit mal patients were reported by Williams in 1950 (Williams, 1953). The interpretation of these findings is difficult because volume conductor effects were not taken into consideration and because of the limited number of cortical and subcortical sites from which the electrical activity was sampled.

Penfield's centrencephalic hypothesis implying a subcortical onset of petit mal seizures was widely, but not universally accepted, after its formulation in 1950. Already in the late forties, some authors claimed a possible cortical origin for the classical generalized and bilaterally synchronous 3/sec spike and wave pattern. This new pathophysiological approach derived from observations carried out in human beings and experimental animals. Hayne et al., (1949) studied a population of 22 epileptic patients with the aid of stereotaxically

implanted electrodes in subcortical structures and simultaneous surface EEG recording. They reported numerous instances in which isolated seizure discharges at a frequency of 3/sec were seen in the cortex with no corresponding discharge in the thalamus or other subcortical regions, Further evidence supporting a cortical origin of the spike and wave pattern was given by Lennox and Robinson (1951) who demonstrated that electrical stimulation of the gyrus cinguli in the cat could produce widespread although predominantly homolateral 3/sec cortical spike and wave activity. Shimizu, Refsun and Gibbs (1952) showed that metrazol injection into one carotid artery in the cat could produce a brief and rather bilaterally synchronous spike and wave discharge recorded from the cortical surface. Vertebral metrazol injections were much less effective in the induction of such a response. The difference in the effectiveness of the two routes of metrazol injection was considered as indicative of a cortical origin of the epileptic discharges. We should mention, however, that more recent information on the distribution of carotid and vertebral blood flow in cats indicates that this assumption may not be valid since it has been shown that the carotid system in these animals supplies the cerebral hemispheres and the thalamus as well (Holmes et al., 1958; Gloor and Testa, 1974). However, Bennett (1953) reported that unilateral intracarotid metrazol injections in petit mal

patients could elicit the electrographic and clinical features of petit mal attacks. Since the thalamus in human beings is usually not irrigated by the carotid system, this observation led him to propose a cortical origin of absence attacks.

These findings were more recently confirmed by Gloor (1968, 1969), who also demonstrated that Metrazol injection through the vertebral route in man, as in the cat (Gloor and Testa, 1974) not only failed to activate bilaterally synchronous spike and wave discharges, but arrested them. Gibbs (1952) also favored a cortical origin of the classical spike and wave pattern at 3/sec observed in petit mal seizures.

The correlation between electroencephalographic and pathological findings in patients with generalized epilepsy led to the discovery that lesions localized to the frontal parasagittal (particularly supplementary motor) medial temporal, or orbito-frontal areas, could cause generalized spike and wave discharges which were indistinguishable from those seen in petit mal epilepsy (Tükel and Jasper, 1952). This phenomenon was called secondary bilateral synchrony. It was postulated that focal cortical discharge could propagate through corticofugal pathways to activate a centrally located neuronal network (the centrencephalic system). The activation of this system would then lead to discharges projected over widespread pathways to both hemispheres, thus inducing generalized spike and wave discharges at a cortical level (Penfield and Jasper, 1954).

Similar results favoring a primary cortical epileptogenic process with a secondary spread of the epileptic activity to subcortical structures was proposed by Starzl et al., (1953) when studying generalized seizures

induced by intravenous metrazol injection to cats. According to these authors the seizures induced by that method originated in the cerebral cortex and then involved deep structures.

The aforementioned arguments supporting directly or indirectly a cortical origin of the spike and wave pattern lead to a reinvestigation of the role of thalamic and brain stem structures in relation to the elicitation of the 3/sec cortical spike and wave pattern. Ingvar (1955) concluded that under suitable experimental conditions it was possible to elicit cortical spike and wave activity during stimulation of the massa intermedia, but that the yield of such responses was low in comparison to the number of stimulations carried out. According to his studies, the elicitiation of spike and wave activity was facilitated if both the intralaminar thalamic system and the mesencephalic reticular formation were stimulated simultaneously. He also reported that under favorable conditions spike and wave responses could be triggered following stimulation of structures located outside the anatomical landmarks assigned to the thalamic reticular system, such as hippocampus and gyrus cinguli. From these experiments Ingvar concluded that the role of a pacemaker in the genesis of generalized spike and wave activity could not be assigned to unique anatomically well defined subcortical structures, as proposed earlier by Jasper (1947). A few years later (1959) Ingvar

reported that spike and wave activity at 3/sec could be recorded in isolated cortical slabs without the need of a subcortical pacemaker, thus clearly favoring a pure cortical origin of the epileptic pattern seen in petit mal.

The role of cortical and subcortical structures in the elicitation of petit mal seizures was reported by Bickford (1956) in an eleven-year-old girl suffering typical absence attacks with characteristic generalized bilaterally synchronous 3/sec spike and wave activity recorded from the electroencephalogram. Stimulation of subcortical structures with deeply implanted electrodes in this patient triggered typical spike and wave discharges at 3/sec which were associated with petit mal attacks. However, similar results were observed to occur after stimulation of widespread cortical areas. This report is interesting because for the first time the possible role of cortical as well as subcortical structures in the elicitation of the patient's seizure pattern (petit mal) was illustrated.

III. Recent observations on the pathophysiology of the spike-wave pattern.

The neurophysiological, pharmacological and clinical electroencephalographic investigations on the pathophysiology of generalized bilaterally

synchronous spike and wave activity carried out during the forties and fifties led to the development of two hypotheses which appeared to be mutually exclusive: one emphasizing the role of subcortical structures (centrencephalic hypothesis) and the other stressing the role of the cerebral cortex (cortical hypothesis).

During the sixties, the interest to understand the pathophysiological mechanisms of the so-called centrencephalic seizures continued to be an outstanding one. Data supporting either the centrencephalic or cortical hypothesis were numerous. Ajmone Marsan et al., (1960) reported that although the association of generalized spike and wave activity with brain tumors was rare, when present, the tumor was predominantly located in the midline, a phenomenon which was considered to be suggestive of a primary brain stem origin of centrencephalic seizures.

Within an experimental context, Guerrero-Figueroa et al., (1963) reported that the application of aluminum oxide into the intralaminar thalamic nuclei and mesencephalic reticular formation in kittens could induce spike and wave discharge in subcortical and cortical structures, provided the animals were younger than 30 days of age. They failed to reproduce their findings in adult cats. Although these authors reported that epileptic changes induced by aluminum oxide application involved subcortical and cortical structures, they did not consider a possible contamination of other subcortical and/or cortical structures with this compound, as it has been suggested in more recent experiments reported by Levin, et al., (1968).

Pollen et al., (1963) and Pollen (1968) reproduced Jasper and Droogleever-Fortuyn's phenomenon but only at "critical levels" of arousal in cats waking from pentobarbital anaesthesia. They also demonstrated that high frequency stimulation of the mesencephalic activating system blocked the spike and wave pattern elicited by 3/sec stimulation of the intralaminar thalamic nuclei, a finding which was at variance with Ingvar's results (1955). Perot (1963) postulated that the spike and wave pattern characteristic of petit mal epilepsy was the result of a condition of abnormal synchrony involving a large number of neurons within the mesencephalic tegmental reticular formation, thalamus and more caudal reticular areas. Further evidence of the role of the mesencephalic reticular formation in the production of spike and wave was provided by Weir (1964) when reporting that generalized spike and wave activity could be elicited following bursts of high frequency stimulation of the midbrain reticular formation at 3 cps in cats. All successful sites of stimulation in his studies fell within the area described by Moruzzi and Magoun (1949) as producting activation of the cortical EEG upon repetitive stimulation. Also, the sites from which spike and wave discharges were produced with the lowest thresholds seemed to be similar to those giving 3 cps waves from aluminum injections in cats (Guerrero-Figueroa et al, 1963).

Also, during this decade neurophysiological studies of the spike and wave pattern concentrated on the morphology of this electrical event (Weir, 1965) and on the correlation of the surface recorded spike and wave activity with unitary activity recorded from the cortex (Weir et al., 1966).

One of the prevailing arguments in favor of a subcortical pacemaker mechanism in centrencephalic seizures has been the elicitation of generalized bilaterally synchronous cortical spike and wave activity following stimulation of the thalamus, combined stimulation of the thalamus and mesencephalic reticular formation or stimulation of the mesencephalic reticular formation alone. The most characteristic EEG pattern of centrencephalic epilepsy is a sudden simultaneous appearance all over the cortex of bilaterally synchronous 3/sec spike and wave discharges emerging from the normal background activity. Such a seizure discharge ends just as abruptly and simultaneously as it started and it doesn't leave signs of postictal depression of cortical activity. The question arises to what extent cortical mechanisms could produce synchronous epileptic discharges without the need of a subcortical pacemaker. Several reports in the literature have supported a cortical origin of generalized epilepsies. Mattson and Bickford (1961) reported an initial disruption in the synchronization of cortical strychnine spikes following section of the corpus callosum in animals. This

finding points towards an evident callosal participation in the synchronization of epileptic discharges in homologous brain regions. However, in their experiments bilateral synchrony of the strychnine spikes was re-established after some time very likely due to a subcortical synchronizing mechanism. Marcus and Watson (1966, 1968) reported an original experimental model of petit mal epilepsy in primates and cats produced by bilateral application of conjugated estrogens to homologous areas of the intermediate frontal region. The bilateral synchrony of the spike and wave discharge induced by the convulsant agent was severely disrupted following section of the corpus callosum. Similarly induced bilaterally synchronous spike and wave activity was recorded in preparations in which all diencephalic, rostral mesencephalic and dorsal hippocampal structures had been ablated. Also, bilaterally synchronous spike and wave activity was elicited, using the same technique, in animals in which large bilateral blocks of cerebral cortex had been isolated from subcortical structures but remained mutually connected by the corpus callosum. Since these original reports, several authors have stressed the importance of the corpus callosum in the synchronization of epileptic discharges following bilateral cortical application of epileptogenic agents (Isaacson et al., 1971; Ottino et al., 1971). The intermediate frontal region also seems to represent the site where the generalized epileptiform discharges occurring naturally in the photosensitive Senegalese baboon, papio papio are first visible (Fisher-Williams et al., 1968; Naquet et al., 1972).

Evidence suggesting that the onset of generalized and bilaterally synchronous spike and wave activity resides in the frontal cortex has been presented by Bancaud et al., (1965), Bancaud (1971, 1974) and Goldring (1972). In the experience of these authors, both the characteristic electrographic pattern of generalized bilaterally synchronous spike and wave discharges and their clinical manifestations could be reproduced by stimulation of the frontal cortex. This type of electroclinical response was not obtained following thalamic stimulation or stimulation of other regions of the cerebral cortex.

Soon after the description of the characteristic EEG pattern occurring in petit mal epilepsy by Gibbs (1935), it was known that the bilateral synchronization of the spike and wave activity both in man and in experimental models of petit mal epilepsy was not perfect (Jasper, 1947; Cohn, 1954; Ogden, 1956; Petsche, 1962; Scarpelezos, 1973). Petsche's observations (1968) that cortical incisions of the cat cerebral cortex extending as far as the white matter resulted in a disruption of the synchronization of epileptic activity led him to propose a role of the cerebral cortex in the synchronization of the epileptic activity.

It is clear from this literature review that both the centrencephalic and the cortical hypothesis proposed to explain the pathophysiology of generalized bilaterally synchronous spike and wave activity during absence attacks received support from clinical and experimental observations. This was surprising, since at first glance the two rival hypothesis seemed to be mutually incompatible.

It was Gloor (1968, 1969, 1972) who attempted to reconcile these two opposing views by assuming the existence of a more diffuse epileptogenic condition involving both the cortex and the reticular system of the higher brain stem and thalamus: the corticoreticular hypothesis. This hypothesis was formulated in 1968 following the observation that in patients with generalized seizures of presumed "centrencephalic" origin, fractionized metrazol injection through one or the other internal carotid artery could reproduce the electrographic and clinical features of a "petit mal" attack. Similarly unilateral intracarotid amytal injection administered during the seizure activity induced by metrazol injection arrested the epileptic activity simultaneously on both sides. In order to understand these findings it is important to realize that the internal carotid circulation in primates including man irrigates the ipsilateral cortex and basal ganglia, while most of the thalamus lies within the territory of the vertebro-basilar circulation (Coceani and Gloor, 1966; Kaplan and Ford, 1966). This means that the thalamic and brain stem diffuse projection systems lie outside the carotid vascular territory and therefore the findings just described could not be explained by an action of the metrazol or amytal on these structures. According to the anatomical distribution of the carotid circulation in man, these substances should be exerting their action at a cortical level. Two patients underwent fractionized intravertebral metrazol injection. In both cases a reduction of the spontaneous generalized cortical epileptic discharges was observed, implying that the brain

stem and/or thalmus exerted an inhibitory effect when activated with an intravertebral metrazol injection. It is also conceivable that some of the inhibitory effect could be due to an activation of the cerebellum since this structure is also irrigated by the vertebral system. Experimental support for the corticoreticular hypotheses, although fragmentary, has come from studies carried out in feline generalized penicillin epilepsy, an experimental model first described by Prince and Farrell (1969) which, in its clinical and EEG manifestations, bears striking similarities with human generalized corticoreticular (centrencephalic) epilepsy (Gloor and Testa, 1974). The earlier work on this model demonstrated that the origin of the generalized penicillin epilepsy in the cat most likely resided in cortical structures (Gloor and Testa, 1974). However, it also signalled the powerful influence of brain stem structures upon generalized penicillin epileptic discharges. Increased desynchronizing drive of brain stem origin induced by intravertebral metrazol injection (Gloor and Testa, 1974) significantly reduced the spontaneous epileptic discharges.

Conversely a reduction in ascending reticular drive induced by mid brain cooling (Testa and Gloor, 1973), or by intravertebral amytal injection (Gloor and Testa, 1974) markedly increased generalized penicillin epileptic activity in cats. The diminution of epileptic discharges recorded from the cortex brought about by increased activity in the ascending reticular formation is probably dependent upon cholinergic mechanisms presumably acting on cortical neurons. Thus intravenous and intracarotid eserine reduced spontaneous epileptic discharges in feline

generalized penicillin epilepsy, while administration of anticholinergic drugs (scopolamine or atropine) increased their incidence (Guberman and Gloor, 1974).

From these studies it appeared unlikely that neurons of the brain stem reticular formation represented a part of the pacemaker mechanism precipitating generalized cortical epileptic discharges. The origin of the abnormal generalized epileptiform activity and the mechanisms involved in their precipitation remained, however, unclarified, although it seems in all probability that structures above the midbrain level, presumably cortex or thalamo-cortical neural circuits were involved in the precipitation of these dicharges (Gloor, 1972).

IV. Anatomical and physiological pathways of the nonspecific thalamic system.

The thalamic nuclei can be divided according to the nature of their thalamocortical projections into: specific and nonspecific thalamic nuclei. The specific thalamic nuclei send thalamo-cortical projections to specific local areas in the cortex which have a systematic topographic localization. Early neurophysiological studies (Dempsey and Morison, 1942, Morison and Dempsey, 1942) demonstrated that stimulation of these nuclei elicited a short latency and localized primary evoked response in the cortex. This short latency evoked response was considered to be mediated by direct thalamo-cortical projection fibres. This hypothesis was confirmed by the presence of retrograde degeneration of

specific thalamic nuclei following cortical ablations or hemidecortication (Walker, 1938; Powell, 1952; Peakcock and Combs, 1965). The nonspecific thalamic nuclei project to widespread cortical areas overlapping the cortical projection areas of the specific thalamic nuclei. Repetitive stimulation of these nuclei elicits a so-called recruiting response (Dempsey and Morison, 1942) or, when the stimulation rate is around 3 c/sec. it sometimes elicits 3 c/sec. generalized spike and wave activity (Jasper and Droogleever-Fortuyn, 1947). The latency of these responses is much longer than the latency of the primary evoked response. Early anatomical studies failed to demonstrate significant retrograde degeneration of the nonspecific thalamic nuclei following cortical ablations or hemidecortication (Nashold et al., 1955). However more recent anatomical studies have shown degeneration of some intralaminar thalamic nuclei after cortical removals in the cat (Murray, 1966).

The original evidence concerning the organization of the nonspecific thalamic projection system was mainly obtained from neurophysiological experiments (Morison and Dempsey, 1942; Dempsey and Morison, 1942; Jasper, 1949; Starzl and Magoun, 1951; Hanbery et al., 1954). The results of these electrophysiological studies indicated that this system functions as a unit and possesses thalamocortical projections which are independent from the thalamocortical projections of the specific thalamic nuclei. This view, however, was not universally accepted (Andersen and Andersson, 1968). It has been proposed that the nonspecific thalamocortical projection system originates in the intralaminar region of the thalamus projecting to the cortex either directly

(Morison and Demsey, 1942) or predominantly by the way of the rostral thalamus (Jasper, 1949, 1960; Starzl and Magoun, 1951; Hanbery et al., 1954; Kao Liang Chow et al., 1959; Weinberger el al., 1965) the rostral thalamic region is according to Skinner and Lindsley (1967) the area through which the "so-called" inferior thalamic peduncle carries projection fibres towards the cortex. Lesions or cryogenic blockades in this area abolish the thalamocortical recruiting response (Skinner and Lindsley, 1967) and also depress the generalized spike and wave discharge elicited by intralaminar stimulation (Villablanca, Schlag and Marcus, 1970).

The first anatomical evidence suggesting a rostrally directed thalamic projection system was presented by Rose et al., (1949) and Nashold et al., (1955). These observations were confirmed later on by the elegant studies of Nauta and Whitlock (1954) and Scheibel and Scheibel (1967). Various anatomical studies have also indicated other possible efferent connections of the intralaminar thalamus such as with the caudate nucleus (Droogleveer-Fortuyn and Stefanis, 1951; Powell and Cowan 1967; Jones and Leavitt, 1974), putamen (Powell and Cowan, 1967) and "rhinencephalon" (Rose and Woolsey, 1949).

Until the seventies, anatomical techniques failed to report conclusive evidence of direct cortical projections from the non-specific thalamic nuclei. However, the orbitofrontal cortex seemed to be an important link in the thalamocortical synchronizing system responsible for spindle bursts and recruiting responses (Velasco, Skinner, Asaro and Lindsley, 1958).

With the aid of retrograde intra-axonal transport of horseradish peroxidase (La Vail et al., 1973), a recently developed anatomical technique, labelling of intralaminar nuclei has been obtained following injection of this enzyme to widespread cortical regions (Jones and Leavitt, 1974). These findings support the existence of nonspecific thalamocortical connections as previously proposed by Murray (1966). Similar findings were reported by Macchi et al., (1975) when describing that after cortical ablations the intralaminar nuclei showed not only chromatolytic changes of their cells but also a true cell loss. These changes were considered to be similar to those observed in the specific thalamic nuclei, thus favoring the hypothesis of direct thalamocortical connections arising from the intralaminar system. Recent anatomical data have shown that the intralaminar thalamic nuclei receive projections arising from the midbrain reticular formation (Bowsher, 1975). These connections could provide the anatomical substratum for the inhibitory effect exerted by the mesencephalic reticular formation upon the nucleus reticularis thalami (Yingling and Skinner, 1975), in the genesis of the desynchronizing effect of MRF activation.

V. Feline generalized penicillin epilepsy

A) Similarities with human petit mal epilepsy.

Generalized penicillin epilepsy in the cat appears to be the most useful animal model presently available to study the presumed pathophysiology of human generalized cortical reticular (centrencephalic)

epilepsy. Its clinical and EEG manifestations are very similar to those seen in the human condition (Prince and Farrell, 1969; Gloor and Testa, 1974). Generalized and bilaterally synchronous bursts of spike and wave activity at a frequency varying between 3 to 4,5 cps are recorded, usually in association with clinical signs such as staring, pupillary dilation, eye blinking, licking, smacking and myoclonic jerks of facial and neck muscles. Furthermore, the electroclinical response to ethosuximide is quite similar to that observed in human patients (Guberman, Gloor and Sherwin, 1975). The responses to intracarotid and intravertebral Amytal and Metrazol injections in feline generalized penicillin epilepsy are the same as those seen in human patients with generalized corticoreticular epilepsy (Gloor and Testa, 1974). The model has the added advantage in that it makes use of a systemic application of an epileptogenic agent which thus comes into contact with all parts of the central nervous system. This reduces the likelihood of an experimental bias in favor of the pathogenetic importance of one versus another set of anatomical structures of the brain as is the case in models in which generalized epileptic discharges are induced by experimental manipulations of the thalamus on one hand or the cerebral cortex on the other.

B) Epileptogenic action of penicillin.

The epileptogenic action of penicillin has been known since Walker, Johnson and Kollros (1945) reported that this drug produced electrographic and clinical epileptiform manifestations both in animals and man, when brought in contact with the brain. Many reports in the literature have since then confirmed this observation (Matsumoto and Ajmone Marsan, 1964 a and b; Prince, 1965; Gloor et al., 1966, Baleydier et al ., 1973; Van Hartesveldt et al., 1974).

The study of the mechanism of action of penicillin at a synaptic level upon cortical neurons aroused the interest of several authors (Matsumoto and Ajmone Marsan, 1964 a, b; Prince, 1965 a, b and 1969; Walsh, 1971; Ayala et al., 1973). It was found (Matsumoto and Ajmone Marsan (1964 a, b)), that cortical neurons responded to penicillin with a sudden, recurrent, abnormally large and prolonged depolarization of the neuronal membrane. These paroxysmal depolarization shifts occurred simultaneously with the electrographic epileptic activity recorded from the surface. More recently Walsh (1971) reported that electrophoretic administration of penicillin near single cortical neuron in cats enhanced the amplitude of certain excitatory synaptic potentials. In spite of an extensive research effort dedicated to understand the epileptogenic action of penicillin, its exact mechanism of action is still unknown. Two main hypotheses have been proposed in order to explain the epileptic properties of this drug. Experiments carried out by Prince (1965 a, b) seem to indicate that the epileptogenic action of this drug may be due to massive release of excitatory transmitter substance by presynaptic terminals leading to excessive and prolonged depolarization of the postsynaptic membrane. More recent experimental works (Curtis et al., 1972, and Davidoff, 1972) have indicated that the epileptic action of

penicillin could be due to a competitive inhibition of GABA, an inhibitory neurotransmitter, occurring post-synaptically at the level of the GABA receptor. The assumption in this hypothesis is that inhibition of an inhibitory mechanism could lead to neuronal excitation.

Although there is overall agreement on the fact that cortical neurons are sensitive to the epileptic action of penicillin, the question whether subcortical structures (thalamic and brain stem structures) are sensitive to the epileptogenic action of this drug, remains a matter of dispute. This difference stands out from previous reports in the literature suggesting that penicillin could induce epileptic discharges when topically applied to any cerebral grey matter located either in cortical or subcortical structures (French, et al., 1955, 1956; Ralston and Langer, 1965; Udvarhelyi and Walker, 1965; Walker and Udvarhelyi, 1965). This view is at variance with the findings reported by Gloor et al., (1966) who demonstrated that thalamic and some extrathalamic structures such as caudate and lentiform nucleus were refractory to the epileptogenic action of penicillin, thus favoring a differential sensitivity of brain structures to the epileptogenic action of penicillin.

VI. Aim of this thesis

From previous experimental work done on feline generalized penicillin epileptic activity (Gloor and Testa, 1974; Testa and Gloor, 1974), it remained unclear whether the epileptic neuronal behavior in this experimental model of epilepsy resides in the cortex, subcortical structures or both. We therefore decided to study the contribution of cortical and subcortical pathophysiological mechanisms in the genesis of feline generalized penicillin epilepsy.

Our experimental results will be presented in three different sections reflecting the sequential order in which we approached the study of the subject.

The first section deals with the role of cortical and subcortical structures in the genesis of feline generalized pencillin epilepsy in animals which received systemic administration of the drug. The experimental evidence presented in this section included EEG recording of generalized penicillin epileptic activity obtained from the cortex and various subcortical structures, as well as the effects of stimulation of cortical and subcortical structures upon the triggering of generalized penicillin epileptic bursts. Such stimulation experiments might identify possible trigger or "pacemaker" mechanisms involved in this model of epilepsy.

The second section deals with the effects of widespread bilateral cortical application of a weak solution of penicillin to the cortical surface of both hemispheres and also with the effect of topical application

of penicillin to different subcortical structures including thalamic and extrathalamic sites. Although Gloor et al., (1966) had demonstrated that most subcortical structures are insensitive to the epileptogenic action of penicillin, their experimental observations had been carried out in anaesthetized animals and it is well known that general anaesthesia markedly interferes with the development of generalized penicillin epilepsy in the cat (Gloor and Testa, 1974). Therefore, we decided to restudy the effect of topical administration of penicillin to subcortical structures in non-anaesthetized animals.

If it could be confirmed that thalamic structures are insensitive to the epileptogenic action of penicillin, this would suggest that the epileptic action of this drug when injected intramuscularly most probably resides in the cortex. If this hypothesis is correct, it should be possible to reproduce the electrographic pattern of generalized penicillin epilepsy in the cat following application of a weak solution of penicillin to widespread areas of the cerebral cortex of both hemispheres.

The third section of our presentation deals with the distribution of radioactive (C14) penicillin in different brain structures following intramuscular, diffuse cortical and topical subcortical application of this drug. In order to define the site of action of penicillin as an epileptogenic agent, it was important to know precisely how this drug was distributed in brain tissue with the different routes of administration and to correlate these findings with the electroencephalographic activity recorded from the cortex and from different subcortical structures.

SECTION I

SYSTEMIC APPLICATION OF PENICILLIN

INTRODUCTION

We decided to study the pathophysiological mechanisms involved in the genesis of generalized spike and wave activity in feline generalized epilepsy, because as already reported in the introduction, this model bears striking similarities with human generalized corticoreticular epilepsy in its clinical and EEG manifestations. (Gloor and Testa, 1974.) Previous experimental work done on this model demonstrated some of the influences exerted by subcortical structures upon generalized epileptic discharges (Gloor and Testa, 1974; Testa and Gloor, 1974; Guberman and Gloor, 1974). From these studies, it appeared that neurons of the brain stem reticular formation exerted an inhibitory effect on the genesis of generalized spike and wave discharge, and they did not seem to represent a part of a pacemaker mechanism precipitating generalized cortical epileptic discharges.

The bilaterally synchronous epileptiform discharges in feline generalized penicillin epilepsy seem to develop gradually out of spindle activity. This phenomenon led us to consider that thalamocortical volleys of neuronal systems involved in spindle generation, could be capable of triggering epileptiform discharges in feline generalized penicillin epilepsy. The aim of the present experimental study was to assess the efficacy of subcortical, especially thalamic structures, on the triggering of generalized penicillin epileptic activity in cats.

MATERIALS AND METHODS

Chronic and acute experiments were carried out in 41 adult cats:

Chronic experiments: In seventeen adult male cats weighing between 3.0-3.7 kg intracerebral electrodes were stereotaxically implanted into various cortical and subcortical structures according to coordinates derived from the stereotaxic atlas of Jasper and Ajmone Marsan (1954). A Kopf stereotaxic frame was used. The intracerebral electrodes consisted of bipolar concentric 24 gauge stainless steel needles with an interelectrode distance of 0.1 mm and a resistance of 40 KΩ. The sites of electrode implantation in chronic experiments are shown in Table I.

The surface EEG was recorded from the cortical convexity by means of 10 stainless steel screws chronically inserted into the skull in bilaterally symmetrical positions over the frontal, central, parietal and occipital brain regions corresponding to the sigmoid gyrus and the anterior, middle and posterior suprasylvian gyri (Fig. 1A). The interelectrode distances were 5 mm. In addition, a reference screw electrode was inserted in the external occipital protuberance. Both the intracerebral and the skull electrodes were connected to a 20 hole Winchester plug which was screwed to the skull and in addition was firmly fixed to it with dental acrylic cement.

All surgical procedures were carried out under aseptic conditions using pentobarbital (Nembutal 30 mg/kg intraperitoneally) as a general anaesthetic. During the operation, single shock and repetitive stimulation was carried out through the intracerebral electrodes in order to

test for the position of the electrodes in the light of electrophysiological criteria. A constant current Nuclear Chicago stimulator was used for these and all other subsequent stimulations. Stimulation of the nonspecific thalamic nuclei triggered barbiturate spindle activity and/ or recruiting responses. Stimulation of specific thalamic nuclei elicited evoked potentials which were recorded only from their corresponding cortical projection areas. The intracerebral electrodes were fixed into their final positions after the desired electrophysiological responses had been obtained.

The experiments on the epileptic condition were begun 7-10 days after surgery at a time when neither the behavior nor the EEG showed any residual effects of the surgical procedure. Bipolar and monopolar EEGs were recorded from the surface and deep intracerebral electrodes on an eight channel Mingograph EEG recording instrument. During the experimental sessions which lasted from 6 to 8 hours, the animals were allowed to move about freely within a well ventilated wooden box (43.5 X 38.5 X 51 cm) with a clear transparent plastic front panel. Most experiments were performed in the same room and the background level of sensory stimulation was kept low in order to promote relaxation of the animals.

Each experimental session consisted of three stages. In the first stage, the background EEG activity was recorded from the surface and the deep intracerebral electrodes for approximately half an hour. In the second stage, single shock and/or repetitive stimulation was performed

through the intracerebral electrodes while the EEG was being recorded simultaneously. At the beginning of the third stage, an aqueous solution of penicillin G sodium was injected intramuscularly in doses ranging from 300,000 to 400,000 I.U./kg. The EEG was then recorded uninterruptedly for 4-6 hours.

Approximately 2 to 3 hours after the penicillin administration when the spontaneous generalized penicillin epileptic activity had reached its peak (Gloor and Testa, 1974; Guberman and Gloor, 1974; Quesney et al., 1975), various cortical and subcortical structures were stimulated in the intervals between spontaneous epileptic bursts through the intracerebral electrodes and the effects upon the EEG, particulary those on the generalized epileptic bursts, were observed. All animals were studied in repeated experimental sessions, but a period of 3 days was always allowed to elapse prior to the next experimental session.

Following completion of the experiments, the animals were deeply anaesthetized with pentobarbital (30 mg/kg intraperitoneally). A 2.0-2.5 ma direct current was applied to each intracerebral electrode for 30 seconds and 10-12 cc of a potassium ferrocyanide solution was injected intravenously. The animals were then exsanguinated and perfused through the heart with 20-30 cc of normal saline and subsequently with 80-100 cc of a 10% formalin solution. The brain was fixed in formalin and 50μ thick histological sections stained with cresyl violet were prepared for verification of the electrode positions.

Acute experiments: Twenty-four cats of both sexes weighing between 2.6-3.6 kg underwent a tracheostomy under ether anaesthesia. Artificial

respiration was started after connecting the tracheostomy tube to an intermittent positive pressure Bird Mark 14 respirator. Halothane at a concentration of 2-2½% was then given as an anaesthetic agent. A wide craniotomy was performed preserving the dura mater intact. Intracerebral electrodes were implanted using the same stereotaxic technique as in chronic animals. The sites of electrode implantation in acute animals are listed in Table I. Following the insertion of the electrodes, the animals were painlessly fixed in a Kopf semichronic head holder through screws attached to the skull. There were no ear or eye bars. Halothane anaesthesia was discontinued at this point and the animals received periodic intravenous injections of small amounts of gallamine triethiodide (Flaxedil, 4 mg/cc) and of fentanyl citrate (Sublimaze, 0.015 mg/cc), a potent narcotic analgesic which does not affect the normal or abnormal EEG (personal observations). The use of this drug together with the painless fixation of the head prevented pain and anxiety. The absence of mydriasis and the abundance of epileptiform discharges after penicillin (which are known to be very sensitive to arousal stimuli) indicated that the animal suffered no discomfort. The expired CO_2 was monitored with a Beckman infrared analyzer. The respiratory rate was adjusted to maintain the CO_2 level at about 4%.

The surface EEG was recorded with silverball electrodes applied against the dura overlying the sigmoid gyri, suprasylvian and lateral sylvian gyri bilaterally. The electrode positions are shown in Fig. 1B.

Various intracerebral structures were electrically stimulated in the same manner as previously described for the chronic experiments. Each experimental session was carried out in three stages as described for the chronic animal experiments. At the end of the experiments, the animals were killed and the brains processed for histological study using the same techniques as used in the chronic animal experiments.

RESULTS

I. Effect of stimulation of cortical and subcortical structures on the EEG, prior to penicillin administration:

(a) Stimulation of nonspecific thalamic nuclei (N.C.M., V.A., oral pole of N. Retic, Pc, C.L.[1]): The effects on the cortical EEG produced by stimulation of the nonspecific thalamic nuclei were consistent and similar in acute and chronic experiments. Under pentobarbital anaesthesia single shock stimulation of the intralaminar nuclei triggered barbiturate spindles bilaterally as originally described by Jasper and Droogleever-Fortuyn (1947). Single shock stimulation of these nuclei in awake animals also triggered stimulus-bound spindle activity which was recorded from the cortex of both hemispheres (Fig. 2A). In awake animals the spindle waves were frequently associated with some slower wave forms which made the spindles look somewhat irregular. Repetitive low frequency stimulation of the nonspecific thalamic nuclei in animals under

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[1] A key to the abbreviations used in this and subsequent sections is to be found in Tables I and II.

pentobarbital anaesthesia induced barbiturate spindle activity bilaterally (Fig. 3A). Similar stimulation carried out in awake animals produced a recruiting type of response which was recorded from the cortex of both hemispheres and also from thalamic and other subcortical structures (Fig. 3B). Spindles or recruiting responses were elicited with such stimulations of the nonspecific thalamic nuclei in 80 to 100% of the trials. In only two animals the phenomenon described by Jasper and Droogleever-Fortuyn (1947) was observed, namely the occurrence of generalized bilaterally synchronous spike and wave activity in response to 2.5-3 cps stimulation of the intralaminar nuclei. These discharges were phase-locked to the frequency of stimulation and did not outlast its end (Fig. 4A).

(b) Stimulation of specific thalamic nuclei (G.M., G.L., V.L., M.D., L.P. and Pulvinar): Single shock or repetitive stimulation of these nuclei evoked unilateral localized potentials which were confined to the cortical projection area of the nucleus which was being stimulated. Spindle activity was not elicited very often (10% or less of the time) and no recruiting responses were seen, except for stimulation of L.P. or of the pulvinar which in some acute and chronic experiments produced a generalized or unilateral response resembling spindle activity or a recruiting response with an incidence ranging from 65 to 100%. It is unlikely that these effects were caused by spread of current to the intralaminar nuclei, since stimulation of other specific or association nuclei (V.L., and M.D.) which are much closer to the intralaminar nuclei did not elicit spindles or recruiting responses. (Fig.5)

(c) Stimulation of extrathalamic sites:

1) Basal ganglia (Putamen, Caudate nucleus and Claustrum). In acute and chronic experiments, stimulation of these structures triggered widespread or generalized responses resembling spindle activity or recruiting responses in 85 to nearly 100% of the trials (Fig. 6A).

2) Other extrathalamic sites: There was only a low incidence of spindle activity and there were no recruiting responses with stimulation of the following structures: surface of the suprasylvian gyrus, orbitofrontal cortex, gyrus proreus, amygdala, hypothalamus, corpus callosum, internal capsule, anterior thalamic radiation and inferior thalamic peduncle. The incidence of spindle triggering for these structures ranged from 0 to 20% of the trials. Low frequency stimulation of the mesencephalic reticular formation was carried out in two acute preparations. In one of these, there was no respone in the EEG, while in the other an ill-defined, spindle-like activity was elicited from both hemispheres with predominance on the side ipsilateral to the electrode implantation in 34% of the trials. Figures 9A and 10A document the incidence of elicitation of spindle activity or of recruiting responses following stimulation of different brain structures in chronic and acute experiments. (The key for the abreviations used in Figs. 9 and 10 is shown in Table II.)

Two clearly segregated groups can be identified: the group on the right comprising the unspecific thalamic nuclei, some basal ganglia, L.P. and the pulvinar exhibits a high incidence of elicitation of spindle

activity or recruiting responses; in contrast, the group on the left comprising all of the other structures shows a low incidence of elicitation of spindle activity and recruiting responses. There is a definite gap between the two groups with no overlap. Only the cingulate gyrus occupies an intermediate position between the two groups with a moderate incidence of spindle triggering (60%).

II. Penicillin-induced generalized epilepsy:

In chronic as well as in acute experiments, the intramuscular injection of high doses of penicillin was followed, as reported previously, by the appearance of generalized and bilaterally synchronous bursts of epileptic activity (Prince and Farrell, 1969; Gloor and Testa, 1974; Guberman and Gloor, 1974; Guberman et al., 1975; Quesney et al., 1975). (Figures 7A and B.) Three main types of bilaterally synchronous epileptic discharges were observed which corresponded to those described by Gloor and Testa (1974): (i) bursts of spike and wave activity at a frequency of 3 to 4.5 cps which fulfilled all the criteria for typical spike and wave complexes as defined by Weir (1965), (ii) bursts of multiple spike and wave activity and (iii) bursts of sharp and slow wave complexes. There was thus some variability of the pattern of the epileptic discharges in the population of animals studied, but each individual animal exhibited a rather consistent discharge pattern throughout one experiment, or from experiment to experiment. The epileptic bursts usually lasted from 2 to 5 seconds. In acute experiments, the spike component was more prominent than in chronic ones, presumably because the EEG was recorded from the dura and not from the skull.

In chronic experiments, generalized epileptic discharges began to appear approximately 30 to 45 minutes after the administration of penicillin; in acute experiments, they appeared only 1 to 2 hours after the penicillin injection, presumably because of a lingering after-effect of the halothane anaesthesia. The peak of the epileptic activity in chronic animals was reached 120 to 180 minutes after the intramuscular administration of penicillin with progressive diminution of the epileptic activity thereafter. The EEG of two animals studied with chronically implanted electrodes was recorded for 24 hours with the aid of an Oxford 4 channel cassette tape recorder (Ives, 1975). No significant epileptic activity was seen 12 hours after a single intramuscular administration of penicillin.

In most animals, generalized paroxysmal EEG abnormalities were recorded simultaneously from the cortex and from subcortical structures. The abnormal electrical activity recorded from subcortical structures was somewhat variable. It assumed a typical or atypical spike and wave form in some instances. This was most often the case in records obtained from the nonspecific thalamic nuclei (N.C.M., oral pole of N, Retic, V.A., P.C., C.L.). The paroxysmal activity in specific thalamic nuclei or in extrathalamic sites consisted mainly of sharp and slow wave complexes or of paroxysmal slow wave activity (Fig. 7A and B). On the basis of our ink written EEGs, these subcortically recorded paroxysmal discharges appeared to be roughly synchronous with those recorded from

the cortex. Both the onset and the end of the paroxysmal activity also tended to be synchronous in cortical and subcortical structures. In a few animals, epileptic activity at its onset was sometimes seen in the cortex only; however, this situation did not last for long. Soon the subcortical structures also became involved. Independent epileptic activity involving only subcortical structures without cortical participation was never observed.

Epileptic bursts in chronic experiments were frequently associated with clinical signs such as staring, eye blinking, pupillary dilatation, licking and myoclonic jerks of the face and neck. In a few animals, generalized tonic clonic convulsive seizures occurred either spontaneously or following several trains of repetitive stimulation of the nonspecific thalamic nuclei. The epileptic discharges increased during drowsiness or sleep as reported earlier (Guberman and Gloor, 1974). In some animals, epileptic bursts were triggered by noise or intermittent photic stimulation.

III. Effect of stimulation of different brain structures on the elicitation of generalized penicillin-induced epileptic activity:

Single shock or low frequency (2.5-8 cps) repetitive stimulation of various cortical and subcortical structures frequently elicited the occurrence of generalized epileptic activity as a stimulus-bound phenomenon. A burst of generalized epileptiform activity was considered to have been triggered by such stimulation if its onset occurred within a

200 msec period after single or repetitive stimulation or at any time during a train of repetitive stimulation. The value of 200 msec was chosen, because it represented the upper limit of the latencies for spindle triggering to single shock thalamic stimulation in Pollen et al.'s (1963) experiments. These stimulation-induced bursts were in all respects similar to those occurring spontaneously. Not all structures were equally effective in precipitating a generalized epileptiform burst in response to stimulation.

In chronic experiments (Fig. 9B) two clearly segregated groups of brain structures can be distinguished with regard to the likelihood with which upon their stimulation generalized epileptiform discharge occurred in the EEG. A t-test shows a statistically significant difference between the two groups ($p < 0.001$). The group on the right side shows a high percentage of triggering of epileptiform activity in response to such stimulation (55 to nearly 100% of the trials). This group comprises the nonspecific thalamic nuclei (N.C.M, V.A., oral pole of N. Retic), some assocation nuclei, (pulvinar, L.P.) and some basal ganglia (Putamen, Caudate nucleus and Claustrum). Figures 2B, 3C, 5B and 6B show examples of such responses. These highly effective structures share another common physiological property: prior to penicillin injection, they all responded to single shock or low frequency repetitive electrical stimulation by eliciting cortical spindle activity or recruiting responses with a high degree of probability (Fig. 9A). In the two animals in which low frequency stimulation of the intralaminar thalamic nuclei

prior to penicillin had elicited bilaterally synchronous spike and wave discharges, the same type of stimulation after penicillin triggered generalized epileptiform bursts which were now no longer phase-locked to the stimulation and outlasted its end. These stimulation-induced bursts after penicillin were entirely similar to the spontaneous epileptic bursts (Fig. 4B).

The group of structures on the left side in Fig. 9B comprises those from which in chronic experiments generalized epileptiform bursts could only be elicited with a low degree of probability (0 to 35% of the trials) (Fig. 8A and B). Prior to penicillin, very little spindle activity and no recruiting responses could be obtained with stimulation of these structures (Fig. 9A). This group includes specific thalamic nuclei (G.M., G.L., V.L.) and extrathalamic sites (Hp., Am., S.S.G., I.C. and C.C.).

The results obtained in acute experiments were similar to those obtained in the chronic ones (Fig. 10B). With regard to the probability of triggering of generalized epileptic activity by single shock or low frequency electrical stimulation, the brain structures which were explored could again be divided into at least two and possible three separate groups. Statistically the group on the right is significantly different from that on the left ($p < 0.001$).

The group on the right side in Fig. 10B comprises structures from which a very high percentage of single shock or low frequency repetitive stimulation resulted in triggering of generalized epileptic bursts (75

to nearly 100%). This group includes the nonspecific thalamic nuclei (N.C.M., V.A., oral pole of N. Retic., Pc and C.L.), but thalamic association nuclei (L.P. and pulvinar) and two basal ganglia (Caudate nucleus and Putamen) are also included in this highly effective group. Prior to penicillin injection, stimulation of these structures elicited the appearance of generalized spindle activity or recruiting responses (Fig. 10A).

The group on the left side is constituted by the structures which when electrically stimulated with single shocks or at low frequency elicited only a low percentage of stimulation-induced generalized epileptic activity (approximately 5 to 30%). This group includes the specific thalamic nuclei (G.M., G.L., V.L., M.D.) and many extrathalamic structures such as Hp, Am, S.S.G, obitofrontal cortex and gyrus proreus, ITP, I.C., M.R.F., A.T.R., and the corpus callosum. Stimulation of these structures prior to penicillin injection elicited little spindle activity and failed to induce recruiting responses (Fig. 10A).

In these acute experiments, two brain structures assumed an intermediate position between the two main groups and may be considered as a separate third group. They included the cingulate gyrus and the mesencephalic reticular formation. The incidence of elicitiation of epileptiform activity by stimulation of these two structures was of the order of 50-55%, but there was no overlap with either the high or the low probability group. Prior to penicillin, at least one of these

structures, the cingulate gyrus, showed an intermediate percentage of triggering of spindle activity upon stimulation (Fig. 10A). It should be noted that high frequency stimulation at 30-60 cps of the mesencephalic reticular formation reduced the incidence of epileptic discharges in one chronic and two acute preparations, a finding which was entirely expected in view of the earlier observations made by Gloor and Testa (1974) and by Testa and Gloor (1974).

The question arises to what extent chance application of electrical stimulation at the time when a spontaneous burst was about to occur may provide spurious evidence for a stimulation-induced response, or in other words, what are the percentage values in Figs. 9B and 10B which would correspond to a chance association of stimulation and occurrence of a spontaneous epileptic burst.

The probability of such a chance association was established in two ways: it was first calculated on a theoretical basis taking into consideration the following features: stimulations were always carried out in between spontaneous epileptic bursts and at a time when the generalized penicillin epilepsy had reached its peak. At that time, there was an average of 4 bursts per minute (Quesney et al., 1975). The duration of each epileptic burst varied from 2 to 5 seconds. Stimulation was not delivered earlier than 1 second after a burst. Therefore, considering each minute of recording time, the real time during which stimulation was delivered varied from 36 to 48 seconds (60 sec -(4 x 6) sec = 36 sec, 60 sec - (4 x 3) sec = 48 sec). Since the burst

frequency was 4 per min, the average available time for stimulation between bursts varied from (36:4) = 9 sec to (48:4) = 12 sec. We had made the assumption that epileptic bursts occurring during repetitive stimulation or within 200 msec following a single shock or the end of a train of repetitive stimulation had been triggered by such a stimulation, the average duration of the trains of repetitive stimulation being 650 msec in both chronic and acute experiments. The probability that an epileptic burst could be recorded merely by chance during or within a 200 msec interval after a period of stimulation can thus be calculated according to the following formula for bursts of 5 or 2 sec duration respectively:

$$\frac{200 \text{ msec} + 650 \text{ msec}}{9{,}000 \text{ msec}} = 0.094 \ (9.4\%) \text{ and } \frac{200 \text{ msec} + 650}{12{,}000 \text{ msec}} = 0.071 \ (7.1\%)$$

On the basis of these calculations, any percentage of association of brain stimulation with the occurrence of a generalized epileptic burst which exceeded 10% was suggestive evidence that at least some of the bursts were stimulation-induced and did not occur by chance.

The probability of chance association of stimulation and epileptic bursts was also established experimentally by the following method: mock stimulations were delivered in the same manner as in the actual stimulation experiments. (A t-test showed that there was no statistical difference in the durations between actual and mock stimulations). In these experiments the output of the stimulator was connected with the

recording instrument in such a way as to produce a stimulus artifact on the EEG record, but no stimulation was actually delivered to the brain. The association between mock stimulations and the occurrence of bursts was determined in the same manner as in the other experiments. Using this method, we established that the probability that stimulation and onset of a burst of generalized epileptic activity would coincide by chance was 8.5%, a figure which is almost identical with the values derived from actual stimulation experiments.

The theoretically and experimentally established probabilities are sufficiently close to allow one to conclude that all values of association of stimulation with a burst which are above 10% of the total number of stimulations given could not be attributed to chance. On this basis, stimulation of the following brain structures were considered to be at least at times ineffective in precipitating generalized epileptiform discharges: Hp, V.L., G.M., G.L., orbitofrontal cortex, S.S.G., C.C., and Am (Figs. 9B and 10B). In all other instances, we must conclude that the various brain structures stimulated must have been capable of triggering generalized epileptiform bursts. This includes some structures (V.L., G.M., G.L., and S.S.G.) which at times proved to be ineffective.

SUMMARY

The mechanism of precipitation of generalized epileptiform discharges in feline generalized penicillin epilepsy, a model of human generalized corticoreticular ("centrencephalic") epilepsy, was studied in acute and chronic experiments in cats with implanted skull and intracerebral electrodes. Single shock and low frequency repetitive stimulation of subcortical sites from which prior to penicillin administration spindle activity and recruiting responses could be elicited, readily triggered epileptiform discharges in the same animals after penicillin. These structures comprised the intralaminar and midline thalamic nuclei, the neostriatum, and some posterior thalamic association nuclei (pulvinar and nucleus lateralis posterior). Subcortical and cortical structures which prior to penicillin elicited neither spindle activity nor recruiting responses were significantly less effective in triggering generalized epileptic bursts after penicillin injection. The probability with which such bursts were elicited from these structures was still, however, in many instances above chance level. It seems therefore that generalized epileptiform discharges in feline generalized penicillin epilepsy can be triggered from a large number of brain sites, but most reliably so from subcortical nuclei involved in spindle generation and recruiting responses. The experimental evidence presented still does not allow one to determine whether epileptic alteration of neuronal function in this form of epilepsy primarily resides in cortical or subcortical nerve cells or in both.

Figure 1: Electrode positions in chronic (A) and acute experiments (B).

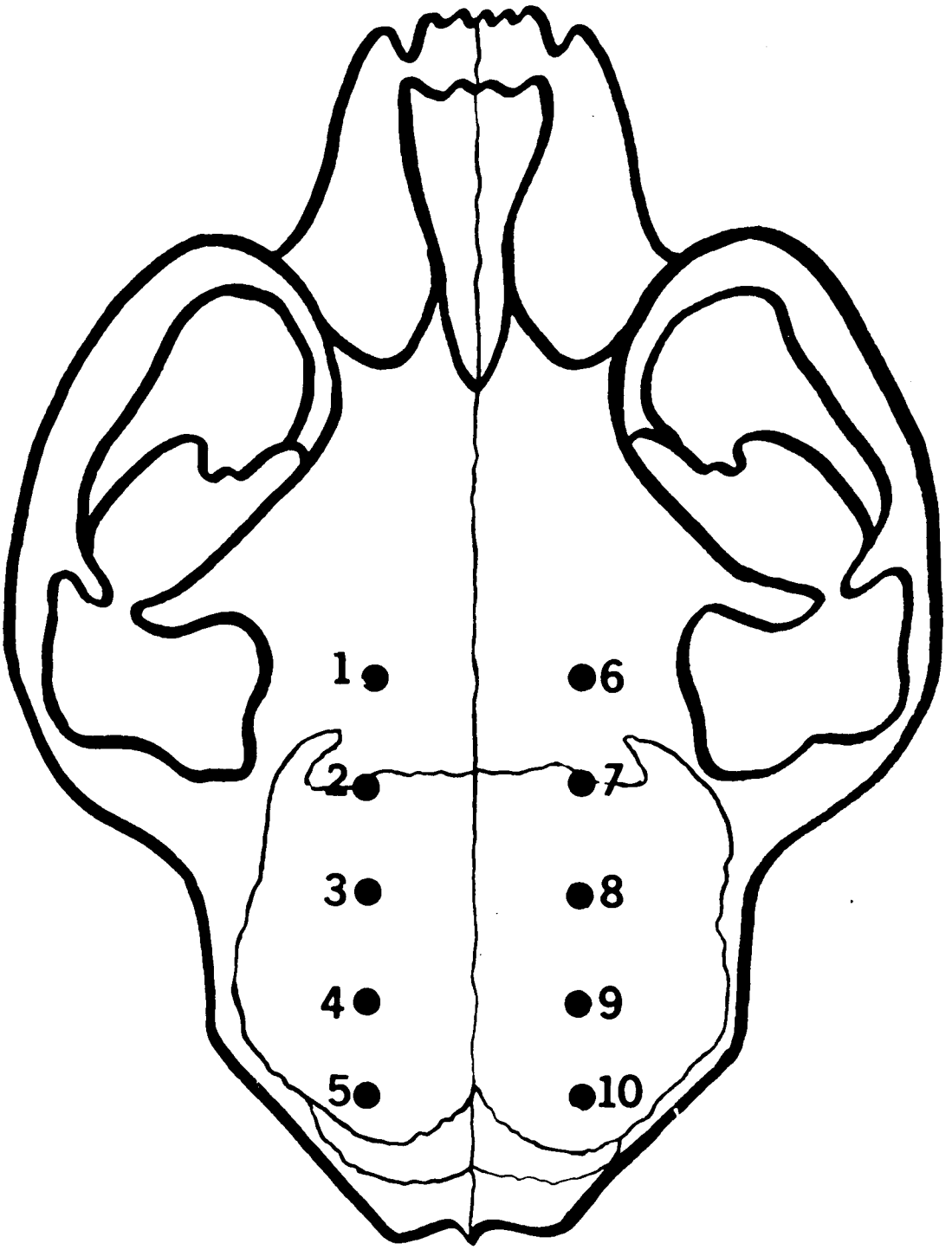


Figure 1A

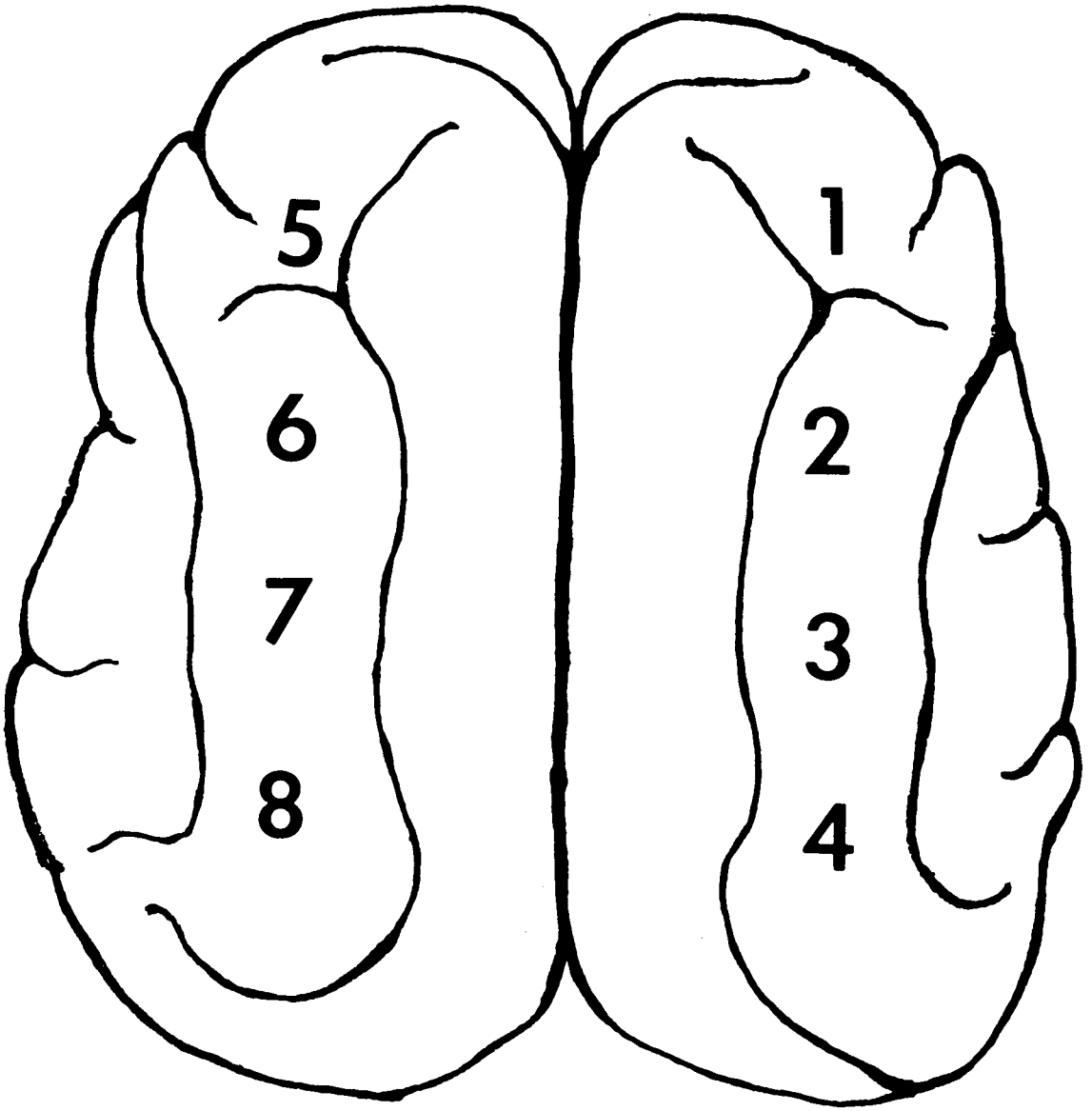


Figure 1B

Figure 2: Stimulation of nucleus centralis medialis (N.C.M.) in the midline prior to and after intramuscular administration of penicillin (Chronic experiment).

A. Elicitation of spindle activity following single shock stimulation of N.C.M. prior to penicillin.

B. Triggering of a generalized spike and wave burst by single shock stimulation of N.C.M. after penicillin.

(In this and subsequent figures, "i" signified intensity, and "f" frequency of stimulation).

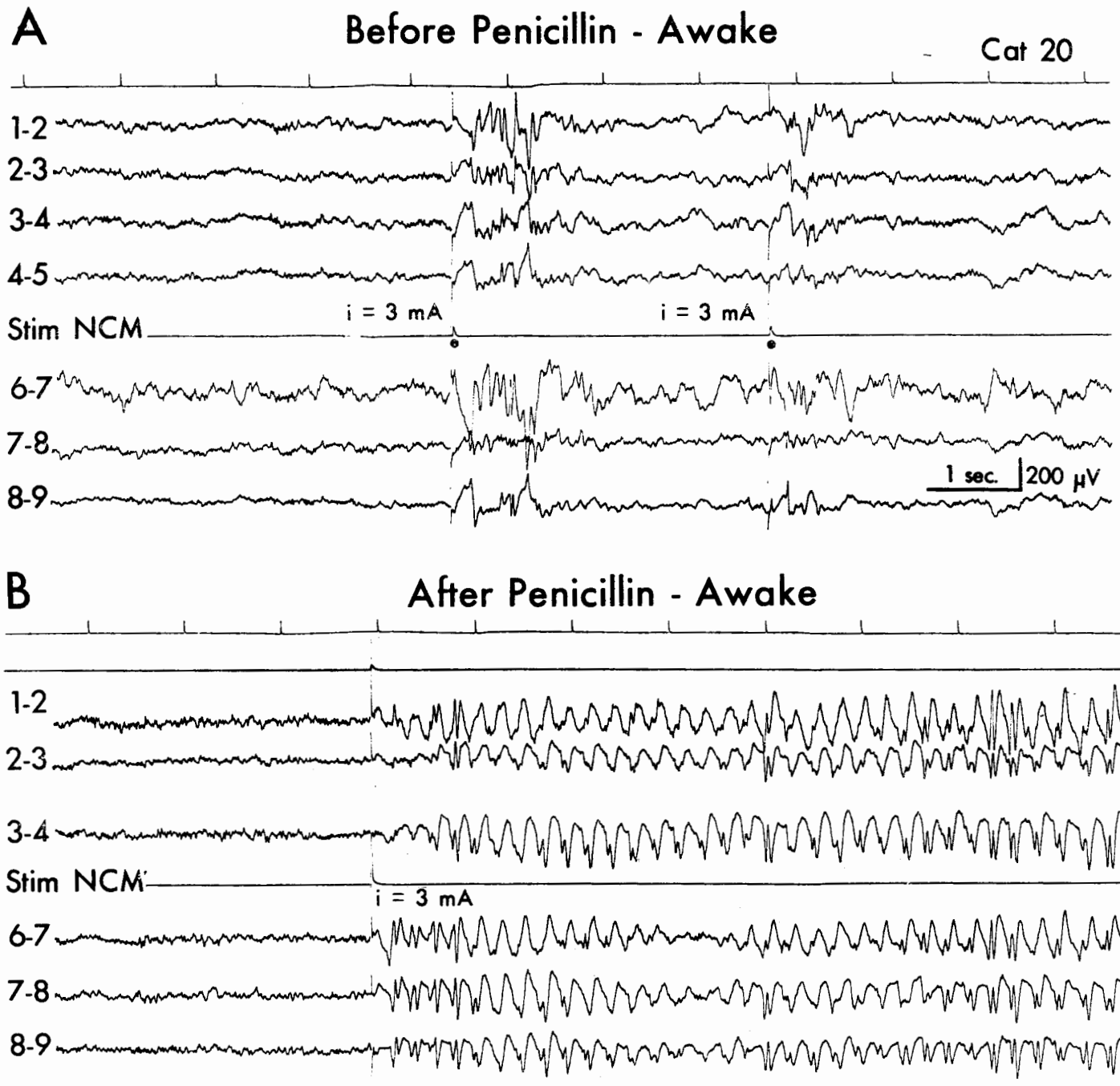

A
Before Penicillin - Awake
Cat 20
1-2
2-3
3-4
4-5
i = 3 mA
i = 3 mA
Stim NCM
6-7
7-8
8-9
1 sec.
200 μV
B
After Penicillin - Awake
1-2
2-3
3-4
Stim NCM
i = 3 mA
6-7
7-8
8-9

Figure 2

Figure 3: Low frequency repetitive stimulation of N.C.M. in the midline prior to and after intramuscular administration of penicillin. (Chronic experiment.)

A. Elicitation of barbiturate spindle activity under Nembutal anaesthesia before penicillin.

B. Elicitation of recruiting response recorded from cortex and subcortical structures before penicillin.

C. Triggering of a generalized spike and wave burst following repetitive stimulation of N.C.M. after penicillin.

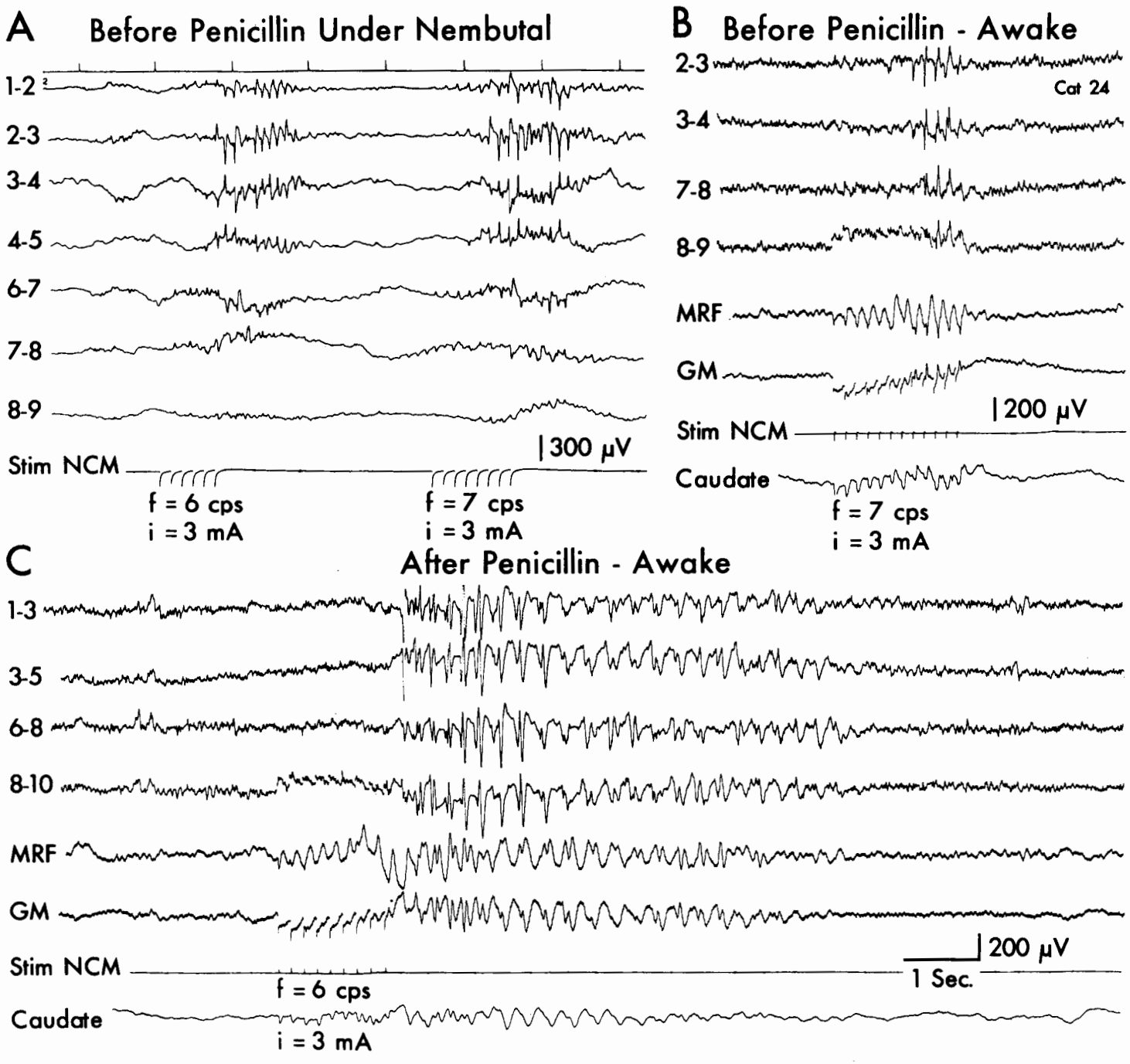

A
Before Penicillin Under Nembutal
1-2
2-3
3-4
4-5
6-7
7-8
8-9
300 μV
Stim NCM
f = 6 cps
i = 3 mA
f = 7 cps
i = 3 mA
B
Before Penicillin - Awake
2-3
Cat 24
3-4
7-8
8-9
MRF
GM
200 μV
Stim NCM
Caudate
f = 7 cps
i = 3 mA
C
After Penicillin - Awake
1-3
3-5
6-8
8-10
MRF
GM
200 μV
1 Sec.
Stim NCM
f = 6 cps
Caudate
i = 3 mA

Figure 3

Figure 4: A. Triggering of generalized bilaterally synchronous spike and wave activity in cortex and in subcortical structures (left V.A., left G.L., and right L.P.) during low frequency stimulation of N.C.M. in the midline prior to intramuscular administration of penicillin.

B. Same as A after intramuscular administration of penicillin. Note that the epileptic discharges now outlast the end of stimulation. A spontaneously occurring generalized epileptic burst is also shown. (Chronic experiment.)

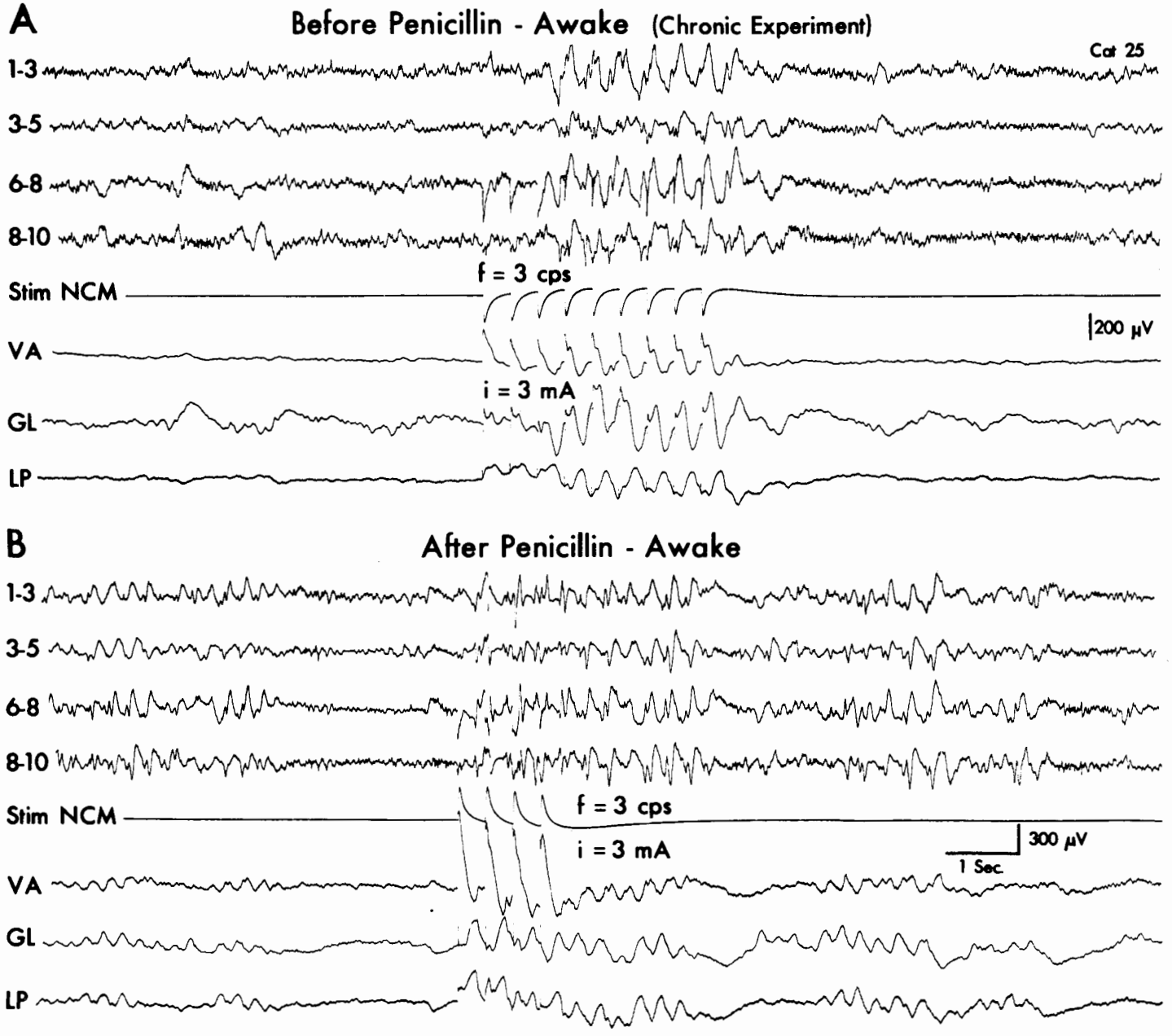

A
Before Penicillin - Awake (Chronic Experiment)
Cat 25
1-3
3-5
6-8
8-10
Stim NCM
f = 3 cps
200 μV
VA
i = 3 mA
GL
LP
B
After Penicillin - Awake
1-3
3-5
6-8
8-10
Stim NCM
f = 3 cps
i = 3 mA
300 μV
1 Sec.
VA
GL
LP

Figure 4

Figure 5: Single shock stimulation of left nucleus lateralis posterior (L.P.) prior to and after intrasmuscular administration of penicillin. (Chronic experiment.)

A. Elicitation of spindle activity following single shock stimulation before penicillin.

B. Triggering of a generalized epileptic burst by single shock stimulation of L.P. after penicillin. A spontaneously occurring generalized epileptic burst is also shown.

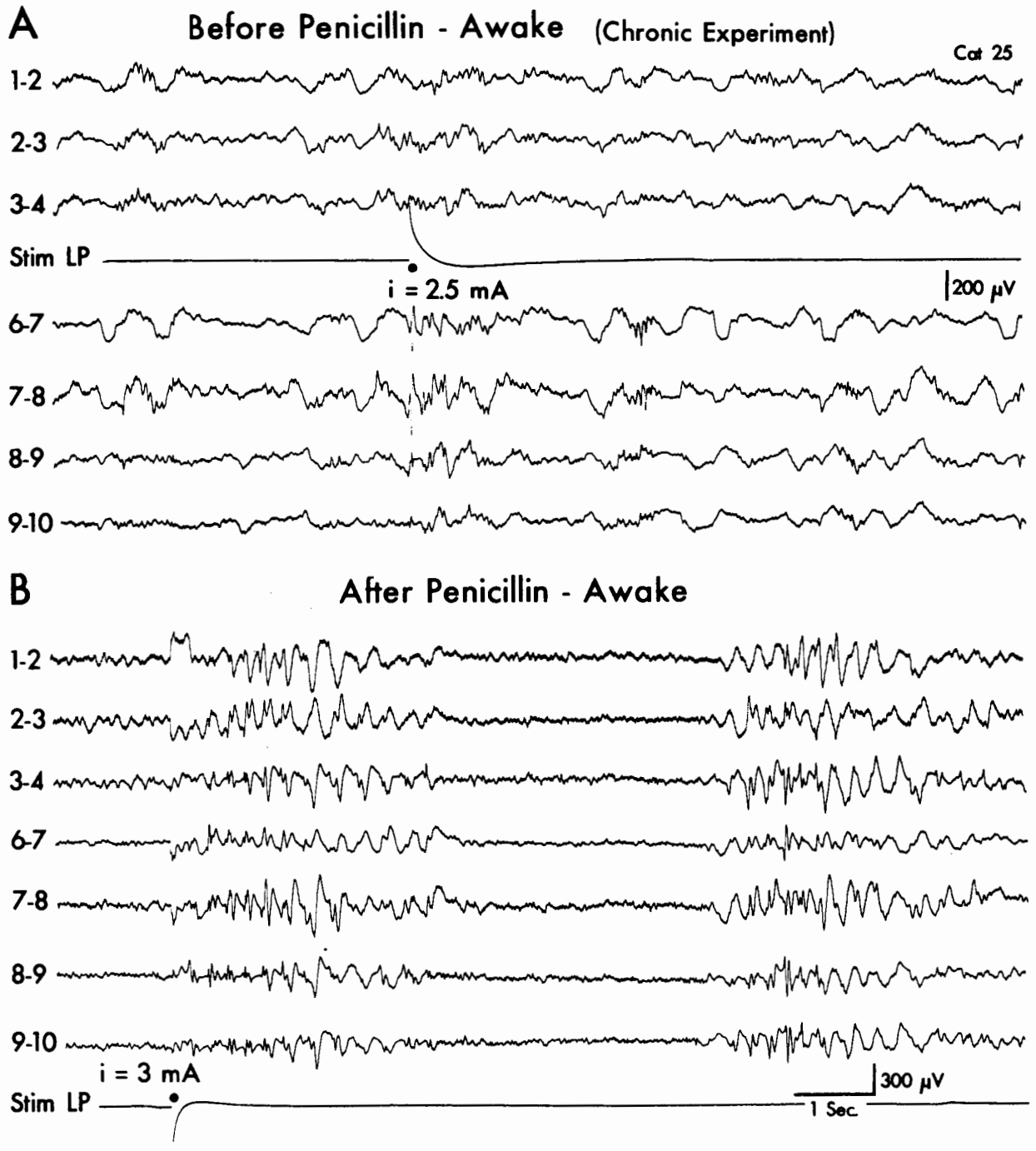

A
Before Penicillin - Awake (Chronic Experiment)
Cat 25
1-2
2-3
3-4
Stim LP
i = 2.5 mA
200 μV
6-7
7-8
8-9
9-10
B
After Penicillin - Awake
1-2
2-3
3-4
6-7
7-8
8-9
9-10
i = 3 mA
Stim LP
300 μV
1 Sec.

Figure 5

Figure 6: Single shock stimulation of left putamen. (Chronic experiment.)

A. Prior to penicillin: elicitation of spindle activity from cortex and subcortical structures.

B. After penicillin: triggering of a generalized epileptic burst recorded from cortex and subcortical structures.

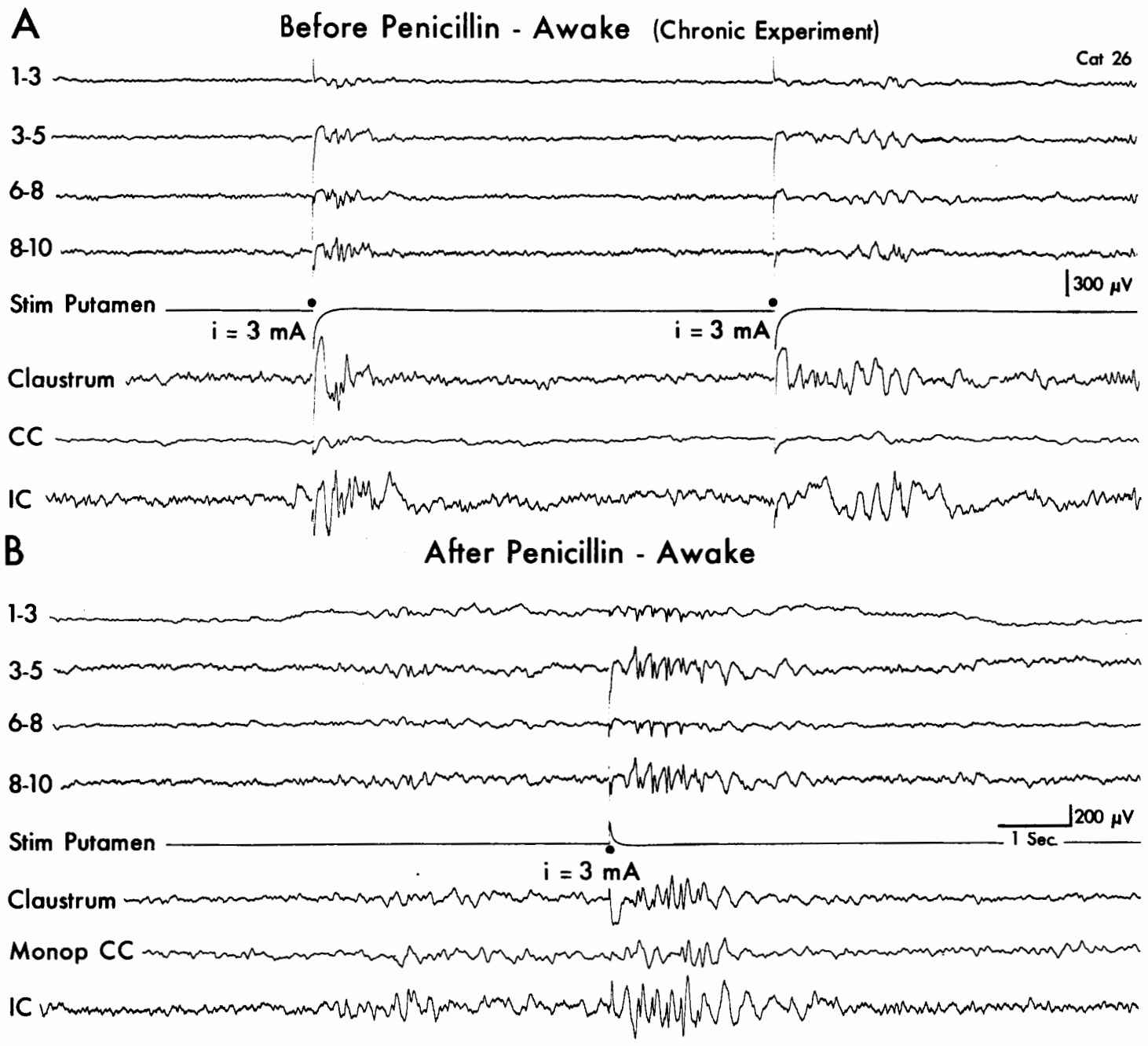

A
Before Penicillin - Awake (Chronic Experiment)
Cat 26
1-3
3-5
6-8
8-10
300 μV
Stim Putamen
i = 3 mA
i = 3 mA
Claustrum
CC
IC
B
After Penicillin - Awake
1-3
3-5
6-8
8-10
200 μV
1 Sec.
Stim Putamen
i = 3 mA
Claustrum
Monop CC
IC

Figure 6

Figure 7: Generalized epileptic activity recorded from cortical and subcortical structures.

A. Chronic experiment (midline N.C.M., left V.A., left G.L., right L.P.).

B. Acute experiment (right N. Retic, left P.C.).

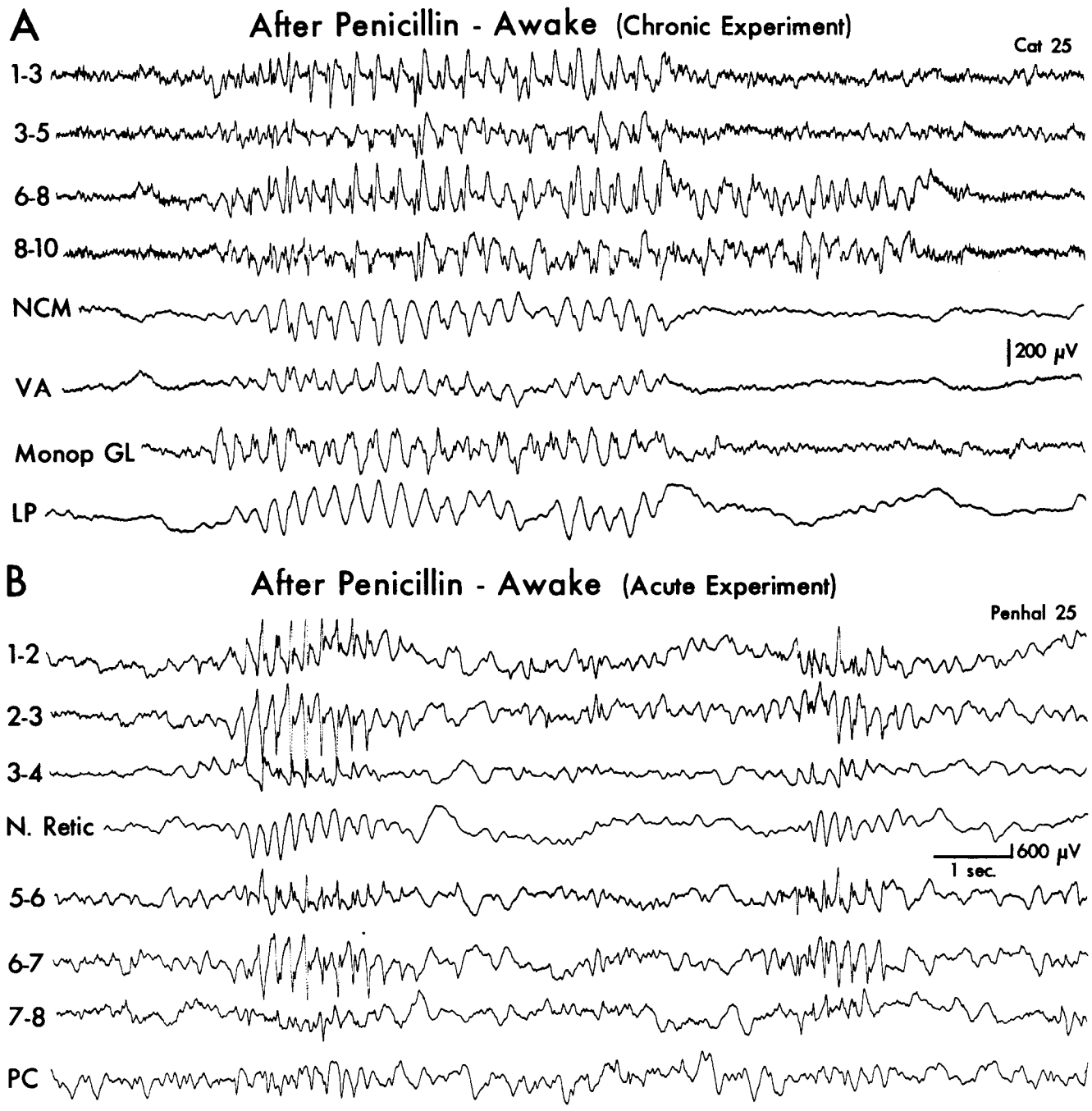

A
After Penicillin - Awake (Chronic Experiment)
Cat 25
1-3
3-5
6-8
8-10
NCM
200 μV
VA
Monop GL
LP
B
After Penicillin - Awake (Acute Experiment)
Penhal 25
1-2
2-3
3-4
N. Retic
600 μV
1 sec.
5-6
6-7
7-8
PC

Figure 7

Figure 8: Failure of triggering generalized epileptic bursts following low frequency repetitive stimulation of left amygdala (Am) (A), or after single pulse stimulation of left medial geniculate body (G.M.) (B). All bursts in this figure are spontaneous.

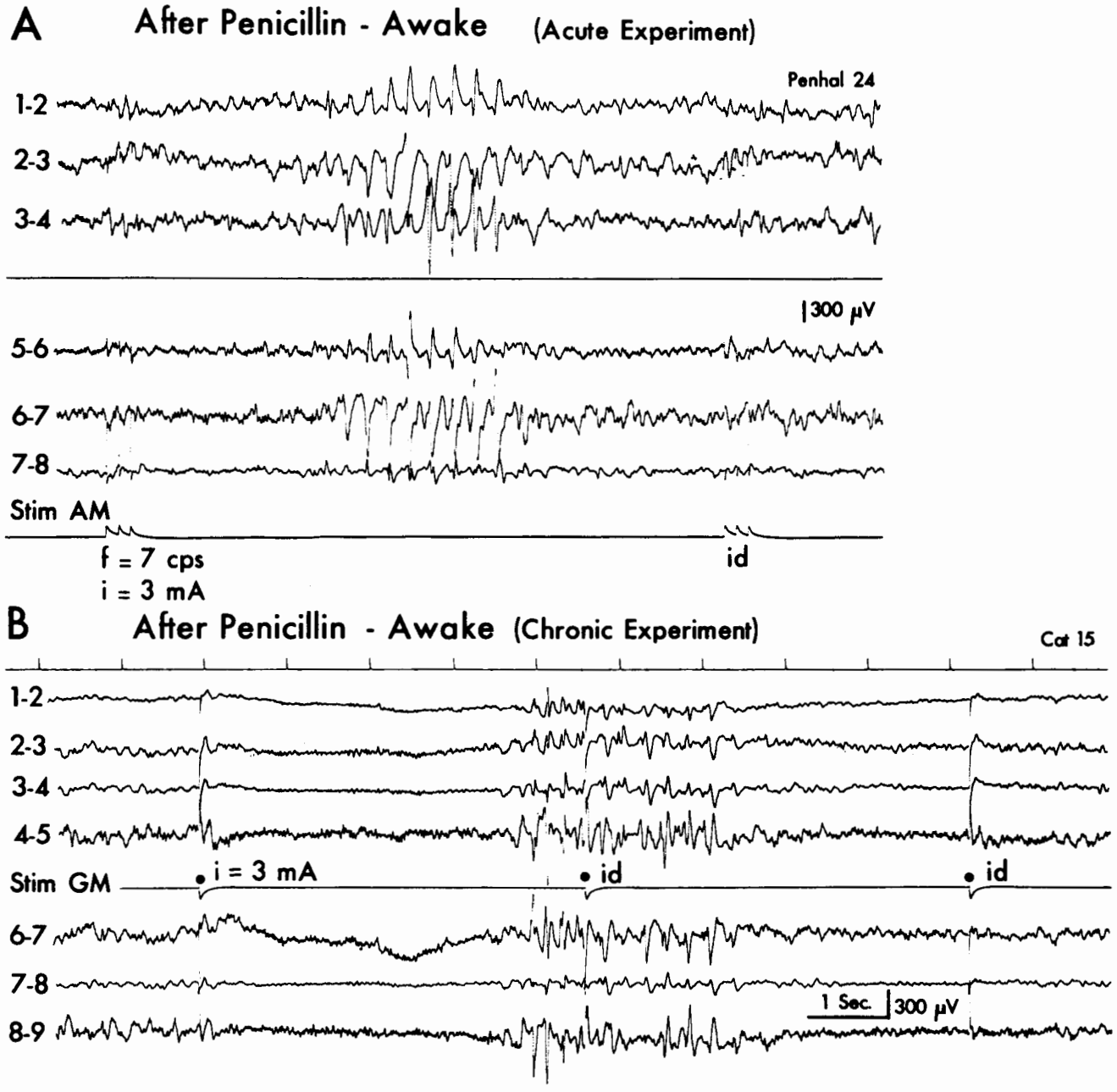

A
After Penicillin - Awake
(Acute Experiment)
Penhal 24
1-2
2-3
3-4
300 μV
5-6
6-7
7-8
Stim AM
f = 7 cps
i = 3 mA
id
B
After Penicillin - Awake
(Chronic Experiment)
Cat 15
1-2
2-3
3-4
4-5
Stim GM
i = 3 mA
id
id
6-7
7-8
8-9
1 Sec.
300 μV

Figure 8

Figure 9: Percentage of trials of single shock and low frequency repetitive stimulation of various brain structures in chronic experiments which elicited spindle activity and/or recruiting responses before penicillin (A), and generalized epileptic bursts after penicillin (B). The various structures are labelled with letters and subscripted numbers which can be identified by referring to the key in Table II. Each symbol represents the average percentage value of several experiments carried out for a single brain structure in a given animal. The vertical arrow indicates the level of chance at which brain stimulation is associated with occurrence of a spontaneous burst of epileptic activity.

Elicitation of Spindle Activity or Recruiting Response Following Stimulation of Cortical and Subcortical Structures Prior to Penicillin (Chronic Experiments)

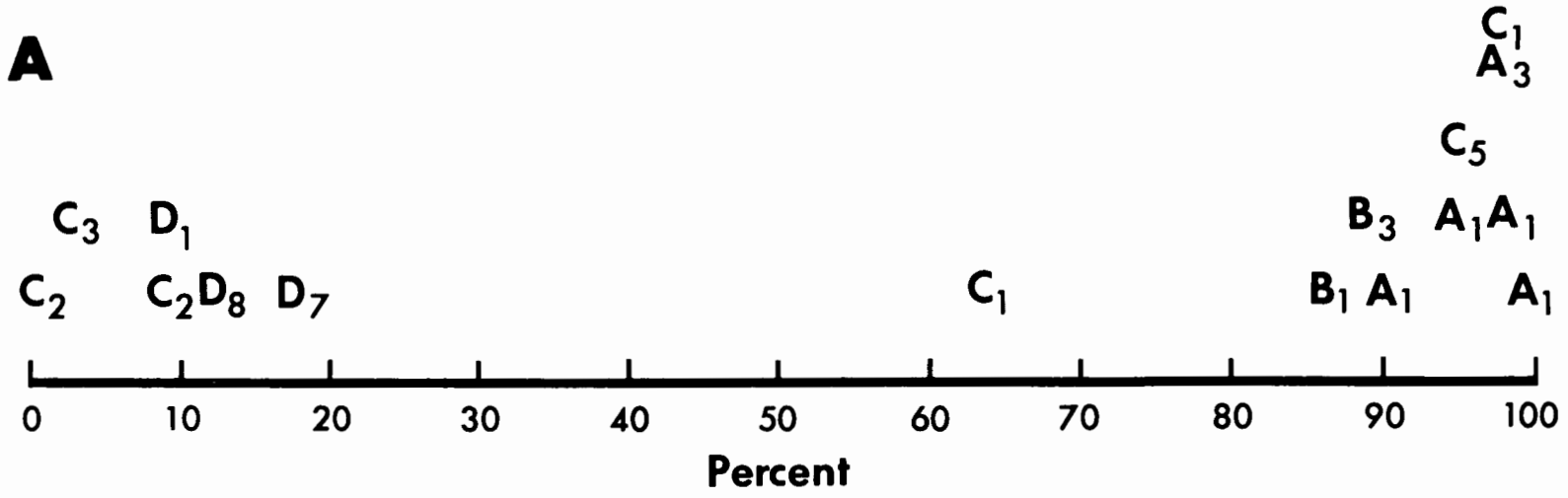


Figure 9

Effect of Stimulation of Different Brain Structures on the Triggering of Generalized Penicillin Epileptic Activity in the Cat (Chronic Experiments)

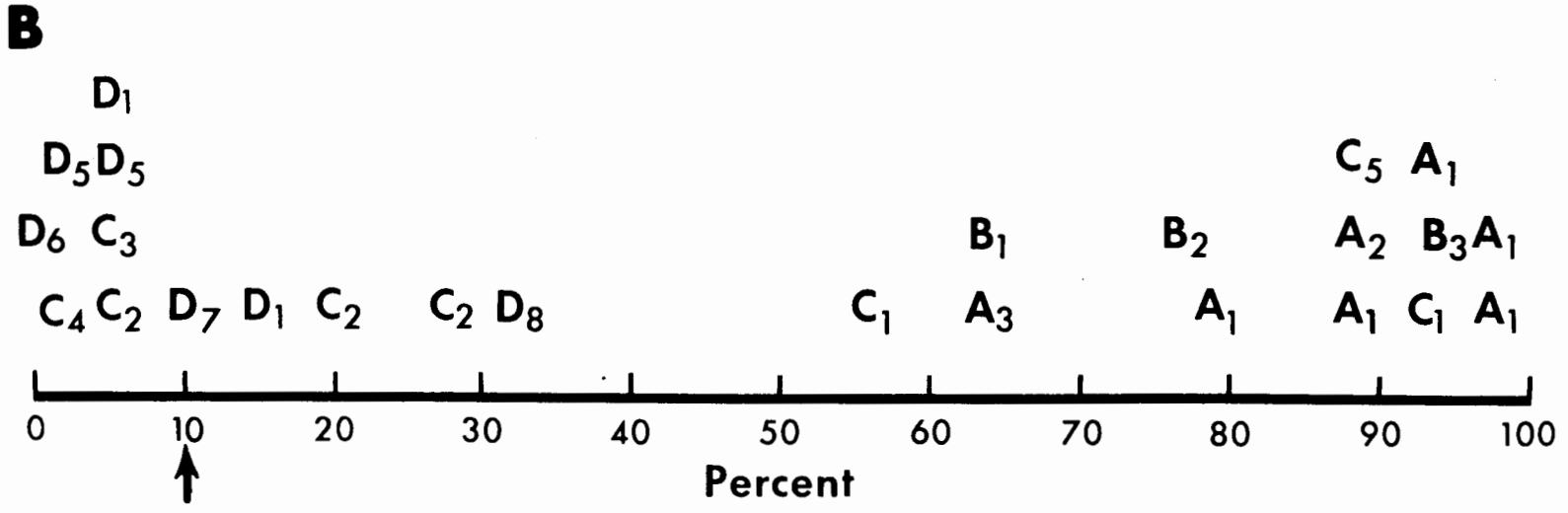


Figure 10: The same as Fig. 7 for acute experiments, except that each symbol represents the percentage value found in a single experimental session for each structure in a given animal.

Elicitation of Spindle Activity or Recruiting Response Following Stimulation of Cortical and Subcortical Structures Prior to Penicillin (Acute Experiments)

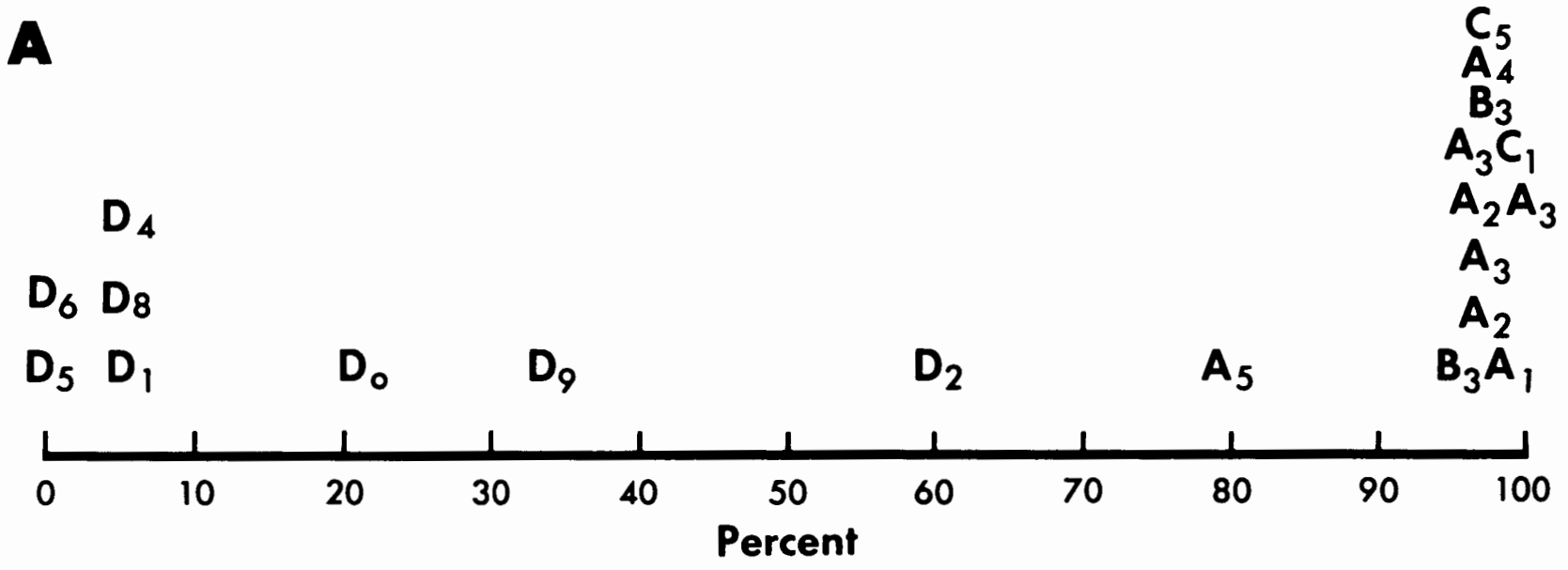


Figure 10

Effect of Stimulation of Different Brain Structures on the Triggering of Generalized Penicillin Epileptic Activity in the Cat (Acute Experiments)

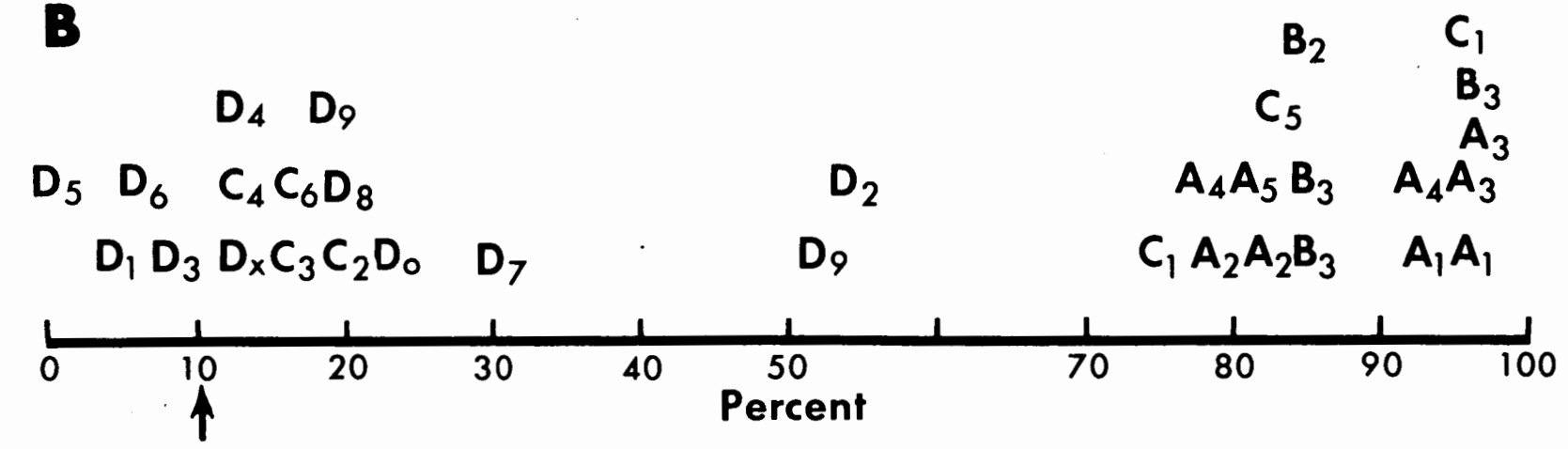


TABLE I

Sites of Electrode Implantation

Anatomical Structure	Chronic Experiments	Acute Experiments
N.C.M. (nucleus centralis medialis including nucleus reuniens)	5	2
N. Retic (nucleus reticularis - oral pole)	1	2
V.A. (nucleus ventralis anterior)	1	2
P.C. (nucleus paracentralis)	0	2
C.L. (nucleus centralis lateralis)	0	1
L.P. (nucleus lateralis posterior)	2	2
G.M. (medial geniculate body)	3	1
G.L. (lateral geniculate body)	1	1
V.L. (nucleus ventralis lateralis)	1	1
Pulvinar	1	1
M.D. (nucleus dorsomedialis)	0	1
Claustrum	1	0
Putamen	1	1
Caudate nucleus	1	3
SSG (suprasylvian gyrus)	2	1
Gyrus cinguli	0	1
Orbito-frontal cortex	0	1
Gyrus proreus	0	1
Am (amygdala)	2	1
Hp (hypothalamus)	1	1
C.C. (corpus callosum)	1	1
I.C. (internal capsule)	1	2*
M.R.F. (mesencephalic reticular formation)	1**	2
A.T.R. (anterior thalamic radiation)	0	1
I.T.P. (inferior thalamic peduncle)	0	1

*A generalized seizure occurred shortly after the onset of I.C. stimulation in one of the animals.

**Only the effects of high frequency stimulation were studied in this animal.

TABLE II

Key to Figures 9, 10 and 12

UNSPECIFIC THALAMIC NUCLEI

A_1 - nucleus centralis medialis (N.C.M.) (including nucleus reuniens)

A_2 = nucleus reticularis (N. Retic - oral pole)

A_3 = nucleus ventralis anterior (V.A.)

A_4 = nucleus paracentralis (P.C.)

A_5 = nucleus centralis lateralis (C.L.)

SPECIFIC AND ASSOCIATION THALAMIC NUCLEI

C_1 = nucleus lateralis posterior (L.P.)

C_2 = medial geniculate body (G.M.)

C_3 = lateral geniculate body (G.L.)

C_4 = nucleus ventralis lateralis (V.L.)

C_5 = Pulvinar

C_6 = nucleus dorsomedialis (M.D.)

EXTRATHALAMIC SITES

Basal Ganglia:

B_1 = Claustrum

B_2 = Putamen

B_3 = Caudate nucleus

OTHERS:

D_1 = suprasylvian gyrus (SSG)

D_2 = gyrus cinguli

D_3 = orbito frontal cortex

D_4 = gyrus poreus

D_5 = amygdala (Am)

D_6 = hypothalamus (Hp)

D_7 = corpus callosum (C.C.)

D_8 = internal capsule (I.C.)

D_9 = mesencephalic reticular formation (M.R.F.)

D_o = anterior thalamic radiation (A.T.R.)

D_x = inferior thalamic peduncle (I.T.P.)

SECTION II

TOPICAL APPLICATION OF PENICILLIN TO THE CEREBRAL CORTEX AND TO SUBCORTICAL STRUCTURES

INTRODUCTION

From the experiments in which penicillin was applied systemically, it remained unclear whether epileptogenic alteration of neuronal activity, presumably responsible for the manifestation of generalized penicillin epilepsy, resided primarily at the cortical, the thalamic or at both levels. It was conceivable that bilaterally synchronous epileptiform discharge was an abnormal, cortical response to otherwise normal thalamocortical volleys, but it was equally conceivable that abnormal thalamocortical volleys induced abnormal discharges in cortical neurons.

The present series of experiments was carried out in order to differentiate between these two hypotheses. Since the epileptogenic action of penicillin on cortex is well documented (Walker et al., 1945; Matsumoto and Ajmone Marsan, 1964a and b; Prince, 1965, Gloor et al., 1966) it was conceivable that the application of a weak solution of penicillin to widespread areas of the cerebral cortex of both hemispheres might reproduce the electrogaphic pattern of generalized penicillin epilepsy in the cat, if the main site of action of intramuscularly injected penicillin in this condition is cortical. Conversely, if an epileptic change in neuronal behaviour in midline thalamic structures were the most important feature in precipitating bilaterally synchronous epileptiform discharges, one would expect that localized application of penicillin to that area of the thalamus should induce generalized bilaterally synchronous epileptiform discharges. This second alternative

seemed less likely, since Gloor et al., (1966) had shown that the local application of penicillin to the thalamus did not produce epileptic discharge, unless there was evidence that the penicillin had escaped to CSF-containing spaces or had diffused to the cortical surface. However, these experiments had been carried out on anaesthetized animals and it was known from an earlier study on feline generalized penicillin epilepsy (Gloor and Testa, 1974) that general anaesthesia markedly interfered with the development of generalized epileptic discharge in this model.

MATERIAL AND METHODS

General procedures: Forty-five cats of both sexes weighing between 2.3 and 3.5 kg were tracheostomized under ether anaesthesia and artificially ventilated with an intermittent positive pressure Bird Mark IV respirator. A wide craniectomy exposing the sigmoid, suprasylvian and lateral sylvian gyri bilaterally was performed under halothane anaesthesia. Intracerebral electrodes were stereotaxically inserted into different cortical and subcortical structures using the coordinates of the stereotaxic atlas of Jasper and Ajmone Marsan (1954). The intracerebral electrodes consisted of bipolar concentric 24 gauge stainless steel needles with an interelectrode distance of 0.1 mm and a resistance of 40 KΩ. Table III lists the sites of electrode implanatation. Following electrode implantation, the head of the animal was painlessly attached to a Kopf semi-chronic head holder without eye or ear bars (for details, see previous section).

Halothane anaesthesia was discontinued. Small amounts of gallamine triethiodide (Flaxedil, 4 mg/ml) and of fentanyl citrate (Sublimaze, 0.015 mg/ml), a potent narcotic analgesic which does not affect the normal or abnormal EEG (personal observations) were injected periodically. An alternation between a waking and sleep pattern in the EEG indicated that the animals suffered no pain or discomfort. The expired CO_2 was monitored with a Beckmann infrared analyzer. The respiratory rate was adjusted to maintain the CO_2 level at about 4%.

The surface EEG was recorded with silver ball electrodes applied to the convexity of both hemispheres in the region of the sigmoid and suprasylvian gyri (Fig. 1B). Bipolar and monopolar EEGs were recorded from the surface and depth electrodes on an 8-channel Elema Schönander Mingograf EEG apparatus.

Single shock and low frequency repetitive stimulation (3-10 cps) was delivered through the intracerebral electrodes using a constant current Nuclear Chicago stimulator.

Diffuse cortical application of a weak penicillin solution (25 cats): Each experimental session had three main stages: First, the background EEG activity from the surface and depth electrodes was recorded for approximately half an hour. In the second stage, single shock and low frequency 3-10 cps repetitive stimulation of the intracerebral electrodes was carried out while the EEG was being recorded simultaneously. In the third stage, a filter paper soaked in an aqueous solution of penicillin G

sodium (50-250 I.U./hemisphere) was applied to the entire exposed surface of each hemisphere. A continuous EEG recording which lasted for 3 to 4 hours was started with the surface electrodes applied against the filter paper overlying the cortical surface. The filter paper was kept in place for a period of 8 to 60 minutes. After removal of the filter paper, the EEG was recorded directly from the exposed cortex. Stimulation of various cortical and subcortical structures was carried out prior to and after the onset of the epileptic activity.

Topical application of penicillin to subcortical structures (20 cats): In each cat of this series, penicillin G in the form of a small crystal or in solution was applied directly to a single or sometimes two subcortical structures with the aid of a stereotaxically driven cannula or with a Hamilton microsyringe (500-1,500 I.U. penicillin in 2-3 µl of solution). Table IV summarizes the anatomical sites to which penicillin was applied in this manner. The EEG was then recorded for approximately 2 hours from the cortex and from the subcortical structures to which penicillin had been applied. In most of the animals of this series, the same dose of penicillin was later also applied topically in the same manner to the cortex in order to demonstrate that the penicillin used was effective in eliciting epileptic discharge.

Anatomical controls: At the end of each experiment of both series, the animals were killed under pentobarbital anaesthesia. Subcortical electrode sites were marked by passing a direct current (2.0 -2.5 mA) for 30 sec. through the intracerebral electrodes. Potassium ferrocyanide was then injected intravenously, the animal exsanguinated after

right atrial section and the brain perfused with normal saline and later with formalin. The locations of the subcortical electrode sites were identified in histological sections stained with cresyl violet.

RESULTS

(1) Epileptic activity induced by cortical application of penicillin.

After diffuse topical application of a weak solution of penicillin (50-250 I.U./hemisphere) to widely exposed areas of both hemispheres, generalized and bilaterally synchronous bursts of epileptic activity developed gradually. This activity was recorded only from the cortex and not from subcortical structures. The pattern of the epileptic activity was similar to that recorded from the cortex of animals which had received high doses of intramuscular penicillin (Figs. 7A and B). Again, three main types of bilaterally synchronous epileptic discharges were observed: (i) Bursts of spike and wave activity at a frequency of 3-4½ cps which fulfilled the criteria of spike and wave complexes as defined by Weir (1965) (Fig. 11), (ii) Bursts of multiple spike and wave activity, and (iii) Bursts of sharp and slow wave complexes.

The pattern of the epileptic discharges was variable from animal to animal, but each individual animal exhibited a rather consistent epileptic pattern throughout the experiment.

The time of onset of the epileptic activity depended on the concentration of the penicillin solution applied to the cortex. With relatively high concentrations (250 I.U./hemisphere), the onset of

bilaterally synchronous bursts of epileptic discharges was early, about 8 to 10 minutes after the topical application of penicillin. With lower concentrations (50 I.U./hemisphere), the onset was sometimes delayed up to 50 minutes. In 3 animals, no spontaneous epileptic activity was ever recorded with these low concentrations. When higher concentrations of penicillin (500-1,000 I.U./hemisphere) were topically applied to both hemispheres, the epileptic pattern consisted mainly of generalized polyspikes and there were also frequent generalized seizure discharges consisting of an initial "tonic" phase with repetitive spike discharges followed by "clonic" phase with repetitive multiple spike and wave bursts.

The epileptic discharges induced by bilateral widespread application of a low concentration of penicillin to the cortex lasted for approximately 2 to 3 hours. The duration of the cortical epileptic bursts varied from 1 to 3 sec. Since the animals were immobilized with gallamine, no clinical signs could be observed.

At no time was abnormal epileptiform activity recorded from subcortical structures either at the time of the cortical bursts or independently from them. However, irregular and low amplitude slow wave activity was occasionally recorded from subcortical sites during cortical epileptic bursts.

(2) Effects of stimulation of various brain structures on the triggering of epileptic activity induced by diffuse cortical application of penicillin:

As had been the case in animals who received penicillin intramuscularly electrical single shock or low frequency repetitive stimulation at 3-10 cps of various cortical and subcortical structures frequently triggered generalized epileptic bursts which were in every aspect similar to those occurring spontaneously. As in the previous study, a burst of generalized epileptiform activity was considered to have been elicited by stimulation if it started within a period of 200 msec after single or repetitive stimulation or at any time during a train of repetitive stimulation. Again, in conformity with the pattern encountered after intramuscular injection of penicillin, two clearly segregated groups of brain structures could be identified with regard to their effectiveness in precipitating generalized bursts of epileptic activity in response to single shock or low frequency stimulation applied to them. The group of structures on the right side of Fig. 12A was highly effective in precipitating generalized bilaterally synchronous epileptic discharge upon such stimulations. The incidence ranged from about 68 to almost 100% of the trials. This population consisted mainly of constituents of the midline and intralaminar system of the thalamus, such as nucleus centralis medialis, the oral pole of nucleus reticularis, nucleus ventralis anterior, but also included the nucleus lateralis posterior and the pulvinar, although stimulation of the latter was definitely less effective

than that of the midline and intralaminar nuclei and of nucleus lateralis posterior. All these structures had in common that prior to penicillin application, they had elicited spindles and/or recruiting waves to single shock or low frequency stimulation with an incidence approaching 100% of the trials (Fig. 12B). The one exception was the pulvinar. Its stimulation, however, was the least effective in producing spike and wave discharge among these highly effective structures. Examples of the responses obtained from stimulation of nucleus centralis medialis are shown in Figs. 13 and 14. In general, single shock stimulation was somewhat less effective than low frequency repetitive stimulation in precipitating these bursts. Frequently, the bursts, especially with the lower concentrations of penicillin applied to the cortex, occurred only for the duration of repetitive stimulation, but in many instances they outlasted its end (Fig. 14).

Quite often, after diffuse cortical penicillin application, stimulation of these highly effective sites in the thalamus was capable of precipitating bilaterally synchronous spike and wave discharges, before there was any sign of spontaneous epileptic discharge. When such discharge later developed, it assumed the same form as that produced by thalamic stimulation. In the 3 animals in which diffuse cortical application of a weak penicillin solution had failed to produce spontaneous epileptic discharges, stimulation of nucleus centralis medialis still elicited bilaterally synchronous generalized epileptic bursts.

In only 2 animals did low frequency stimulation of the intralaminar nuclei at 2½-3 cps trigger generalized bilaterally synchronous spike and wave activity before application of penicillin. This activity did not significantly outlast the end of stimulation (Fig. 15A). After diffuse cortical penicillin application, similar spike and wave discharges were triggered at a time when no spontaneous epileptic bursts were seen, but the effectiveness of thalamic stimulation in inducing these bursts was now much greater than before penicillin, since the intensity of stimulation required to elicit such a response was about 10 times lower than prior to penicillin application (Fig. 15B).

The population of highly effective sites for the precipitation of bilaterally synchronous epileptiform activity after diffuse cortical application of penicillin was in one respect different from that found in the animals of our earlier study in which the penicillin had been given intramuscularly. Thus stimulation of the caudate nucleus, although it was highly effective in precipitating spindle activity or recruiting responses (as had been the case in earlier experiments with intramuscular penicillin), in contrast to that earlier study, showed no particular effectiveness in precipitating spike and wave discharges after diffuse cortical application of penicillin. In one animal, repetitive stimulation of the caudate nucleus failed to precipitate typical epileptic bursts, but in 30% of the stimulations, bursts of high voltage bilateral sharp waves resembling spindles were produced which outlasted the end of stimulation. In another animal, the incidence of triggering epileptic bursts was only 25%.

On the left side of Fig. 12A, the structures are shown which upon single shock and low frequency stimulation yielded only a very low incidence of precipitation of generalized epileptic bursts. The incidence of precipitation of epileptic bursts ranged from 0-25% of the trials. The chance level of coincidence of stimulation with spontaneous occurrence of a burst was calculated in the present study with the method used in the previous study and was found to be 2.7%. (The difference between this figure and the 10% figure found in the previous study is explicable, since some of the experimental data which enter into the calculation of probability were different in this series of experiments. Thus the burst frequency was 1.4 per min., the average burst duration was 1.2 sec., and the average duration of the trains of repetitive stimulation was 930 msec.). Assuming that any value of 3% or more denotes an association between a burst and a train of stimulation which is better than chance, it becomes apparent that although relatively ineffective, stimulation of some of the structures on the left side of Fig. 12A yielded responses above chance level. The gyrus proreus, amygdala, hypothalamus and the corpus callosum performed barely above chance level. The medial and lateral geniculate nuclei, the internal capsule and the caudate nucleus were somewhat more effective. The structures in which stimulation was not effective in precipitating epileptiform bursts included the claustrum, putamen and the mesencephalic reticular formation. All of these structures had a very low effectiveness in producing spindle

triggering or inducing recruiting responses as is seen in Fig. 12B. The observation that stimulation of putamen and claustrum failed to produce spindles or recruiting responses in this series of animals differs from that made in the previous study with intramuscular injection of penicillin but the correlation between the absence of precipitation of spindles or recruiting responses before penicillin and the lack of effectiveness of such stimulations in producing generalized bursts of epileptic discharges after penicillin is in line with our earlier observation showing a close relationship between these two types of electrophysiological responses.

(3) Effects of topical administration of penicillin to subcortical structures:

The subcortical structures to which penicillin was applied topically either in crystal form or by microinjection (500-1,500 I.U.) are listed in Table IV. No epileptic activity was ever recorded from thalamic or extrathalamic subcortical structures to which penicillin was thus applied (Fig. 16), with the exception of the amygdala (Fig. 17) where spike discharges promptly occurred after local penicillin application. No cortical discharges, either focal or generalized, were ever seen after such subcortical penicillin applications. In order to make sure that negative effects were not caused by some failure of the epileptogenic potency of the penicillin used, the same amount of penicillin was later in the experiment applied to the cortical surface in most animals of

this series. Invariably upon local cortical application, focal epileptic spike activity soon appeared in the cortical area where penicillin had been applied (Fig. 17B).

Topical application of penicillin to subcortical structures, although it failed to induce epileptogenic changes, nevertheless changed the cerebral electrical activity in some ways: frequently it induced bursts of slow waves from the structures to which it had been applied and sometimes the cortical EEG became more desynchronized.

SUMMARY

In the cat, topical applications of a weak penicillin solution to large areas of cortex of both hemispheres (50-250 I.U./hemisphere) induces bilaterally synchronous epileptiform discharges, often of a characteristic spike and wave type, which in all respects are similar to those obtained with intramuscular injection of high doses of penicillin. The only difference is that with diffuse cortical application no synchronous paroxysmal high voltage discharges are seen in subcortical structures. This cortically induced generalized epileptiform condition responds in the same manner as that induced by intramuscular injection of penicillin to single shock and low frequency stimulation of a variety of brain structures: bilaterally synchronous epileptiform discharges are precipitated with a high probability by stimulations in those subcortical structures which in the normal animal easily induce spindle waves or

recruiting responses (intralaminar and midline nuclei of the thalamus and some association nuclei); in contrast, stimulation of other cortical and subcortical structures which are much less effective in inducing spindles and recruiting responses, are also less effective in precipitating bilaterally synchronous epileptiform discharges. Topical application of penicillin to subcortical sites, particularly to the thalamus, including the intralaminar and midline nuclei, fails to elicit any form of epileptic discharge (except for the amygdala).

TABLE III

Sites of Electrode Implantation

Structure	No. of Experiments
N.C.M. (nucleus centralis medialis)	8*
N. Retic (nucleus reticularis - oral pole)	2
V.A. (nucleus ventralis anterior)	1
L.P. (nucleus lateralis posterior)	1
G.M. (medial geniculate body)	1
G.L. (lateral geniculate body)	1
Pulvinar	1
Claustrum	1
Putamen	1
Caudate nucleus	2
Gyrus proreus	1
Am (amygdala)	1
Hp (hypothalamus)	1
C.C. (corpus callosum)	1
I.C. (internal capsule)	1
M.R.F. (mesencephalic reticular formation)	1

*Two of these animals do not appear in Figure 12 because stimulation before penicillin application already produced bilaterally synchronous spike and wave discharges.

LG: Lateral gyrus: SSG: middle suprasylvian gyrus: ESG middle ectosylvian gyrus.

For key to other abbreviations, see Table III.

TABLE IV

Topical administration of Penicillin G to different cortical and subcortical structures

Experiment Identification	Subcortical Structure	Cortical Structure
1	NCM	-
2	NCM	-
3	NCM	-
5	NCM	-
6	NCM	-
7	NCM	LG
8	NCM	SSG
10	NCM	SSG
11	NVM	SSG
12	NCM MRF	SSG
13	NCM	SSG
14	NCM	SSG
15	NCM	SSG
16	NCM	SSG
17	NCM	SSG
18	Am	-
19	Am	-
20	Caudate G.M.	SSG
21	VA G.M.	LG
22	V.L. N. Retic	ESG

Figure 11. Spontaneous generalized spike and wave burst induced by diffuse cortical application of a weak penicillin solution. (In this and subsequent figures, N.C.M. indicates midline nucleus centralis medialis of the thalamus.)

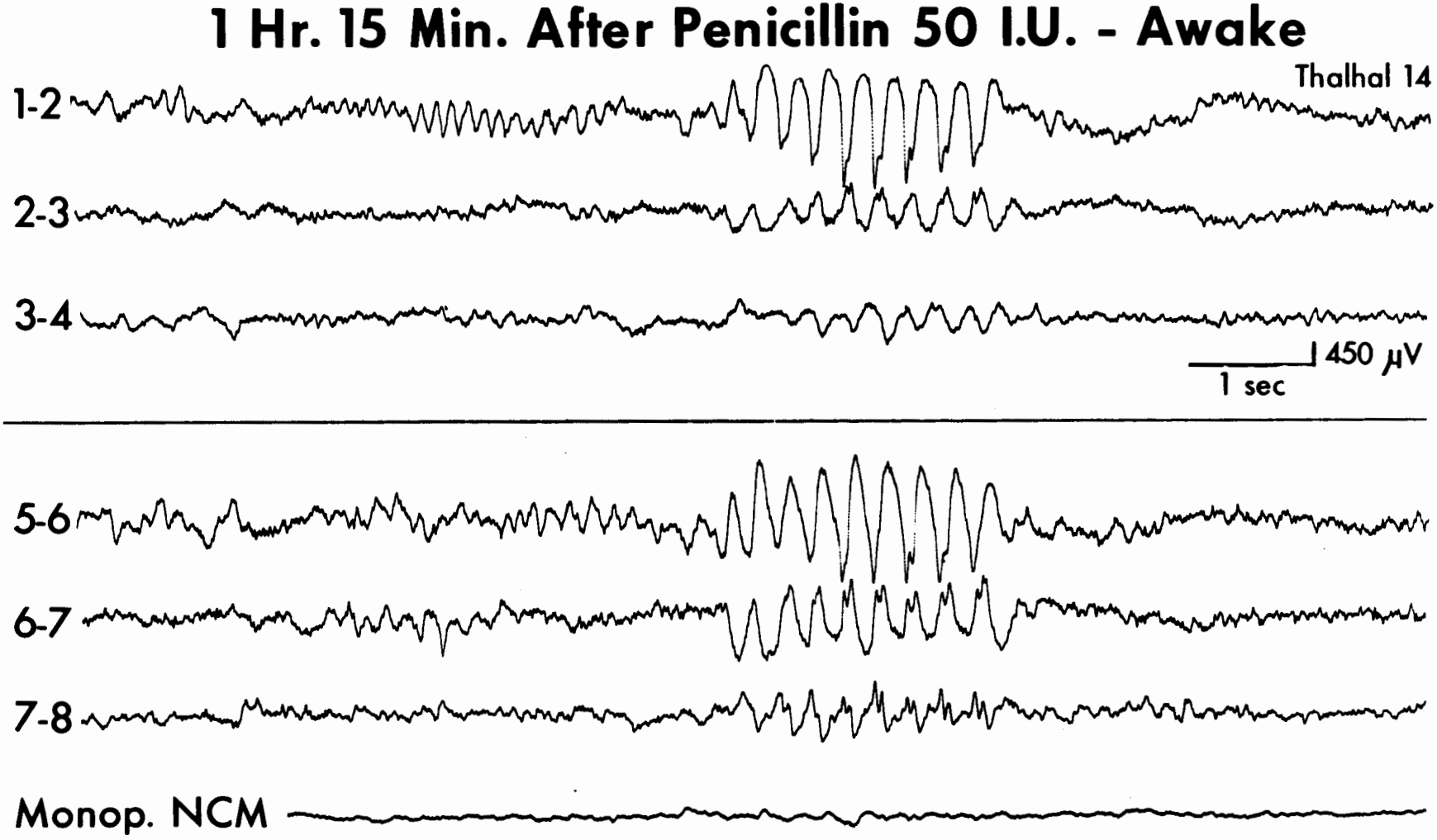
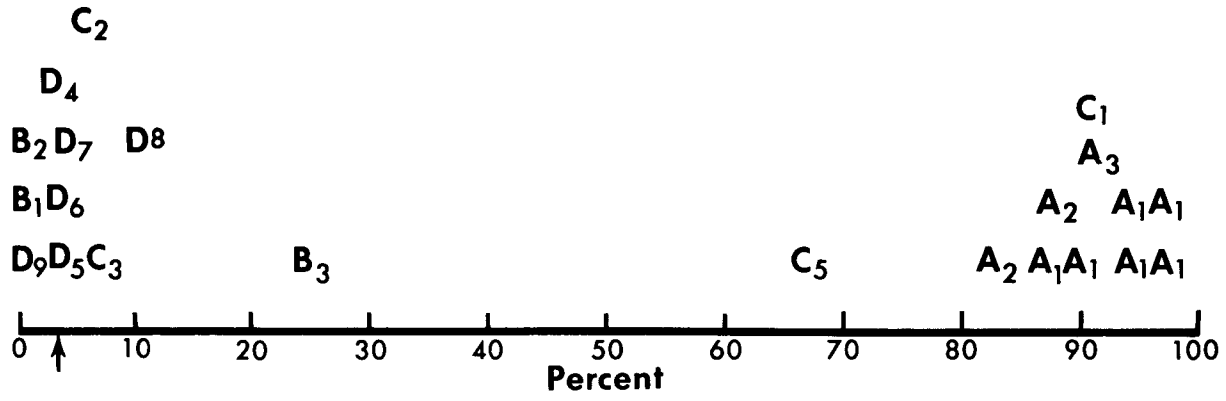

1 Hr. 15 Min. After Penicillin 50 I.U. - Awake
Thalhal 14
1-2
2-3
3-4
450 μV
1 sec
5-6
6-7
7-8
Monop. NCM

Figure 11

Figure 12. Percentage of elicitation of generalized epileptic bursts after diffuse cortical application of penicillin (A), and of spindle activity and/or recruiting responses before penicillin application (B), by single shock and low frequency repetitive stimulation of various brain structures. The structures labelled with letters and subscripted numbers can be identified by referring to the key in Table III. Note: in (B) two animals with stimulation of the caudate nucleus (B_3) are listed, but only one appears in (A) because in one of these animals (B_3 at 85% in B) stimulation after penicillin did not produce definite bursts of epileptic activity, but only bilateral sharp waves resembling spindles.

A

Effect of Stimulation of Different Brain Structures on the Triggering of Epileptic Activity Induced by Cortical Application of Penicillin



B

Elicitation of Spindle Activity or Recruiting Response Following Stimulation of Cortical and Subcortical Structures Prior to Cortical Application of Penicillin

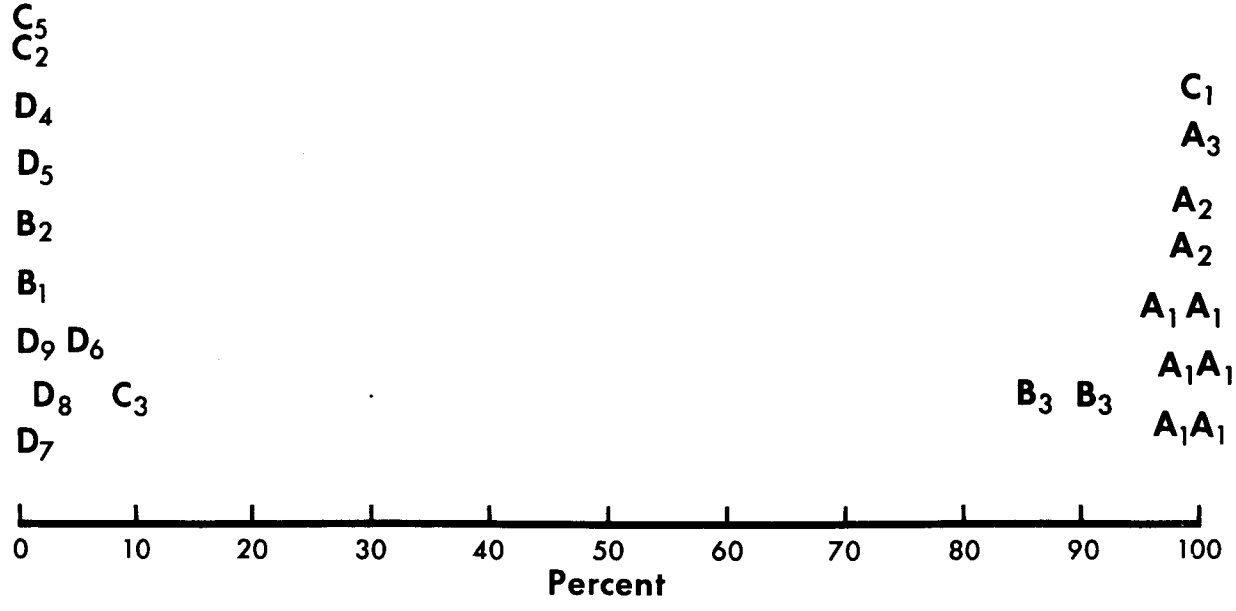


Figure 12

Figure 13. A: Before penicillin application: 7 cps stimulation of N.C.M. elicits a recruiting response.

B: After diffuse cortical penicillin application (50 I.U./ hemisphere) in the same animal: 4 cps stimulation of N.C.M. elicits bilaterally synchronous spike and wave activity.

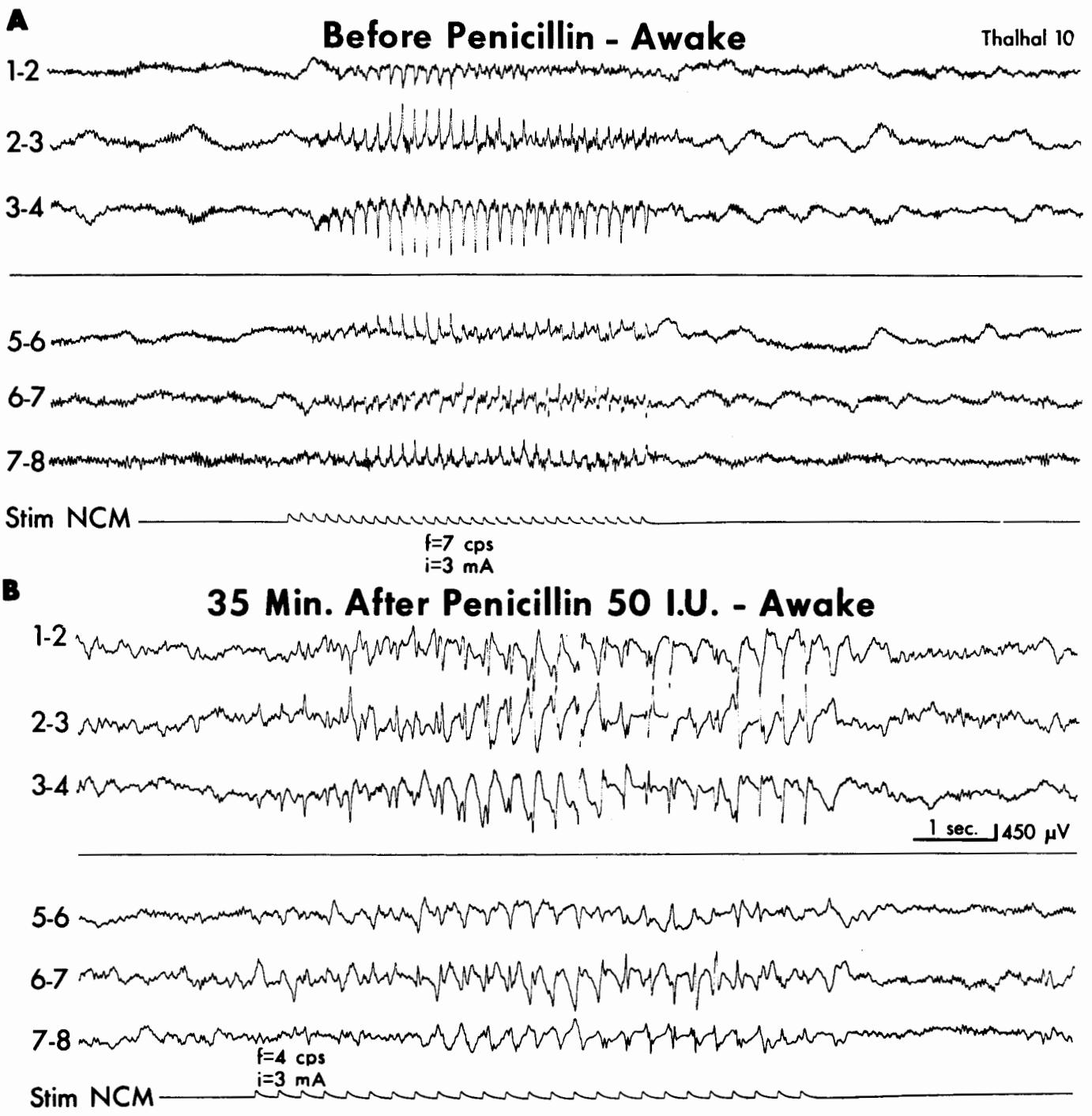
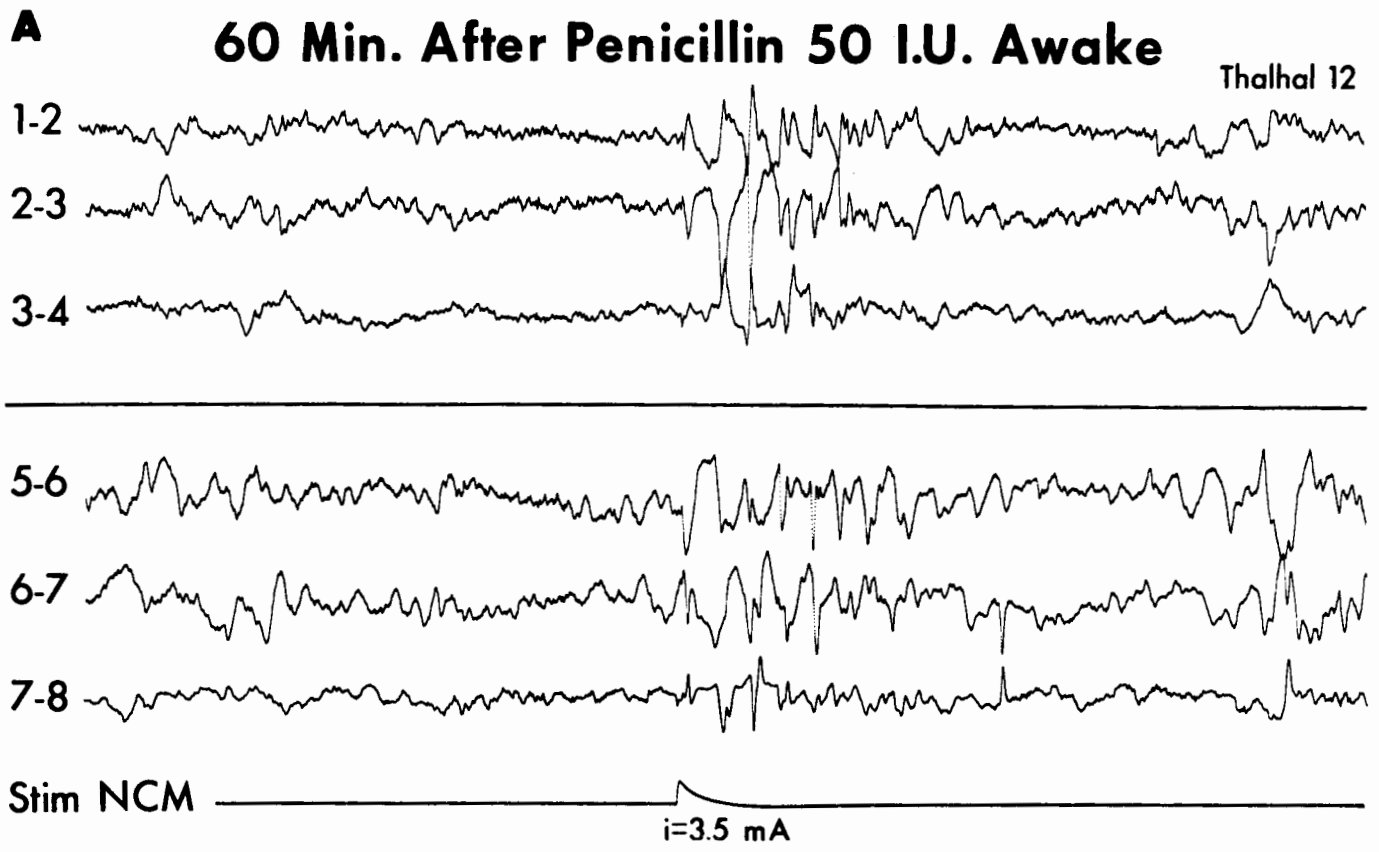

A
Before Penicillin - Awake
Thalhal 10
1-2
2-3
3-4
5-6
6-7
7-8
Stim NCM
f=7 cps
i=3 mA
B
35 Min. After Penicillin 50 I.U. - Awake
1-2
2-3
3-4
1 sec.
450 µV
5-6
6-7
7-8
f=4 cps
i=3 mA
Stim NCM

Figure 13

Figure 14. Effect of single shock (A) and 6 cps repetitive stimulation (B) of N.C.M. in 2 different animals after diffuse cortical application of penicillin (50 I.U. in A, 250 I.U./hemisphere in B). In both A and B the induced epileptiform burst outlasts the period of stimulation.


A
60 Min. After Penicillin 50 I.U. Awake
Thalhal 12
1-2
2-3
3-4
5-6
6-7
7-8
Stim NCM
i=3.5 mA

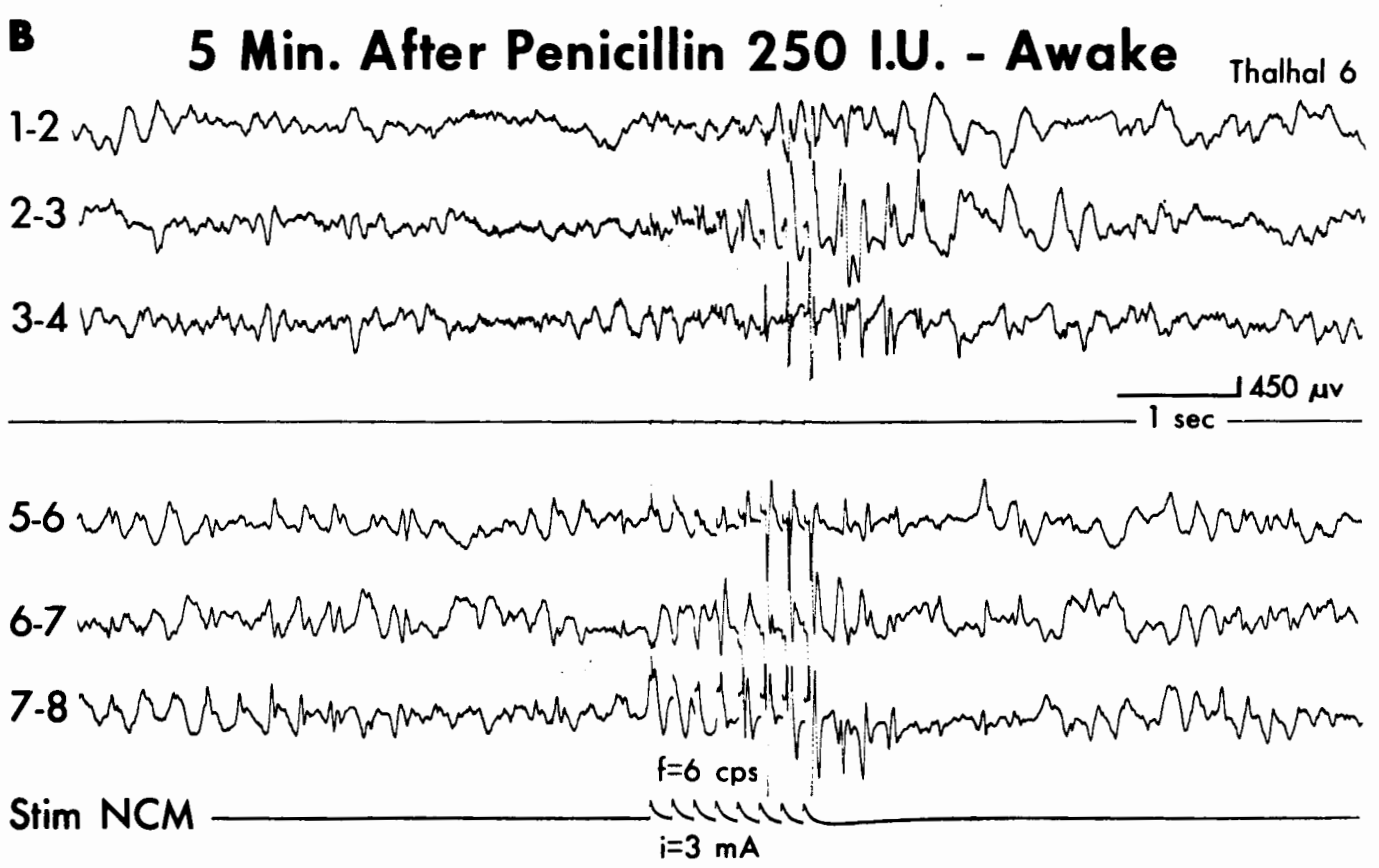

B
5 Min. After Penicillin 250 I.U. - Awake
Thalhal 6
1-2
2-3
3-4
450 µv
1 sec
5-6
6-7
7-8
f=6 cps
Stim NCM
i=3 mA

Figure 14

Figure 15. A: Bilaterally synchronous spike and wave activity elicited by 3 cps repetitive stimulation at 3 ma in a cat before penicillin application (Jasper-Droogleever Fortuyn (1947) phenomenon).

B: Same animal after diffuse cortical application of penicillin (50 I.U./hemisphere): Large bilaterally synchronous spike and wave discharges are now elicited with 4 cps N.C.M. stimulation with an intensity of only 0.3 ma.

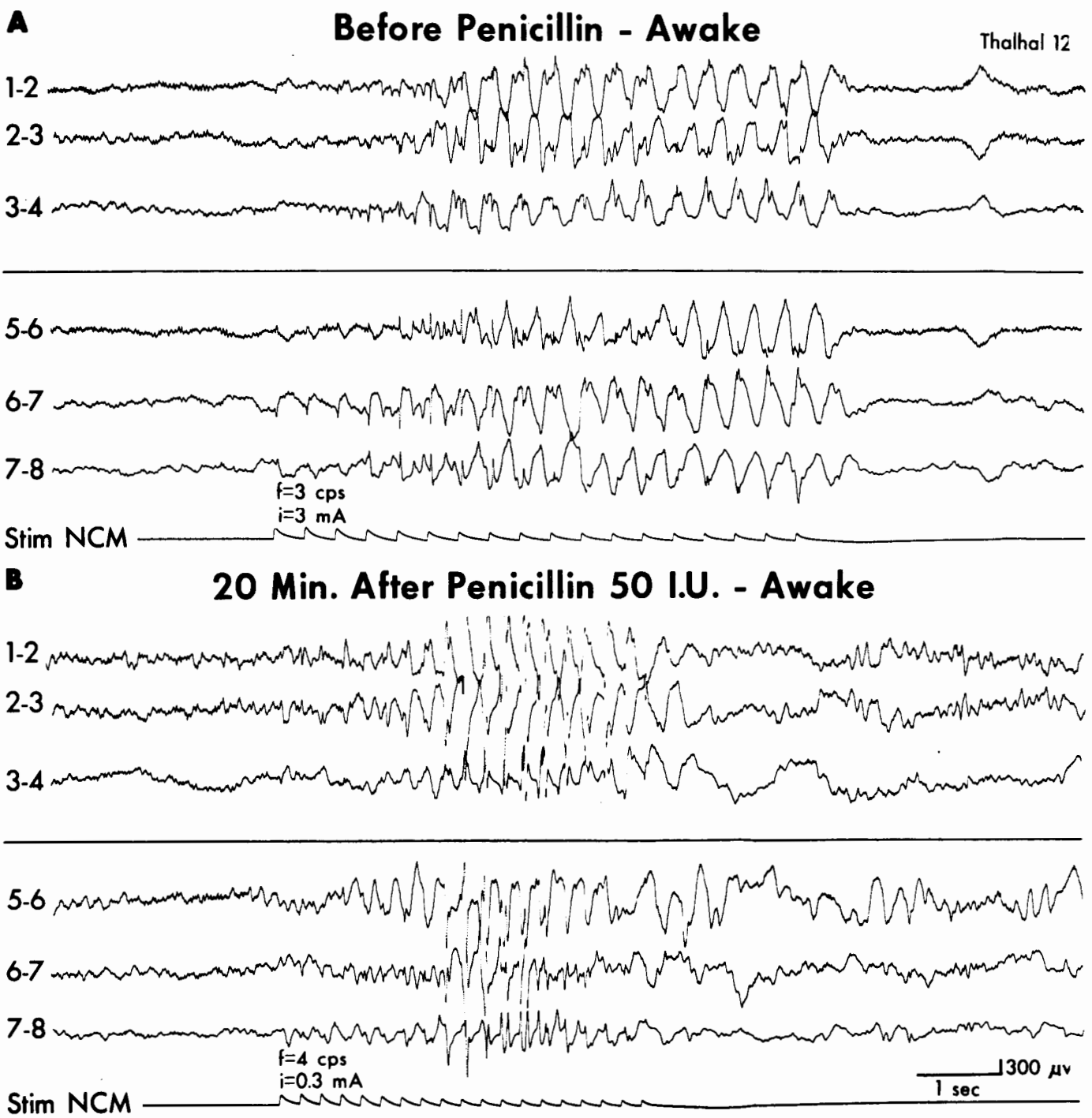

A
Before Penicillin - Awake
Thalhal 12
1-2
2-3
3-4
5-6
6-7
7-8
f=3 cps
i=3 mA
Stim NCM
B
20 Min. After Penicillin 50 I.U. - Awake
1-2
2-3
3-4
5-6
6-7
7-8
f=4 cps
i=0.3 mA
300 µv
1 sec
Stim NCM

Figure 15

Figure 16. A: Cortical and thalamic (left nucleus ventralis anterior, V.A., and right lateral geniculate body, G.L.) recording before application of penicillin.

B: The same recordings 2 hours after application of a penicillin crystal to V.A., and 30 minutes after a similar application to G.L.

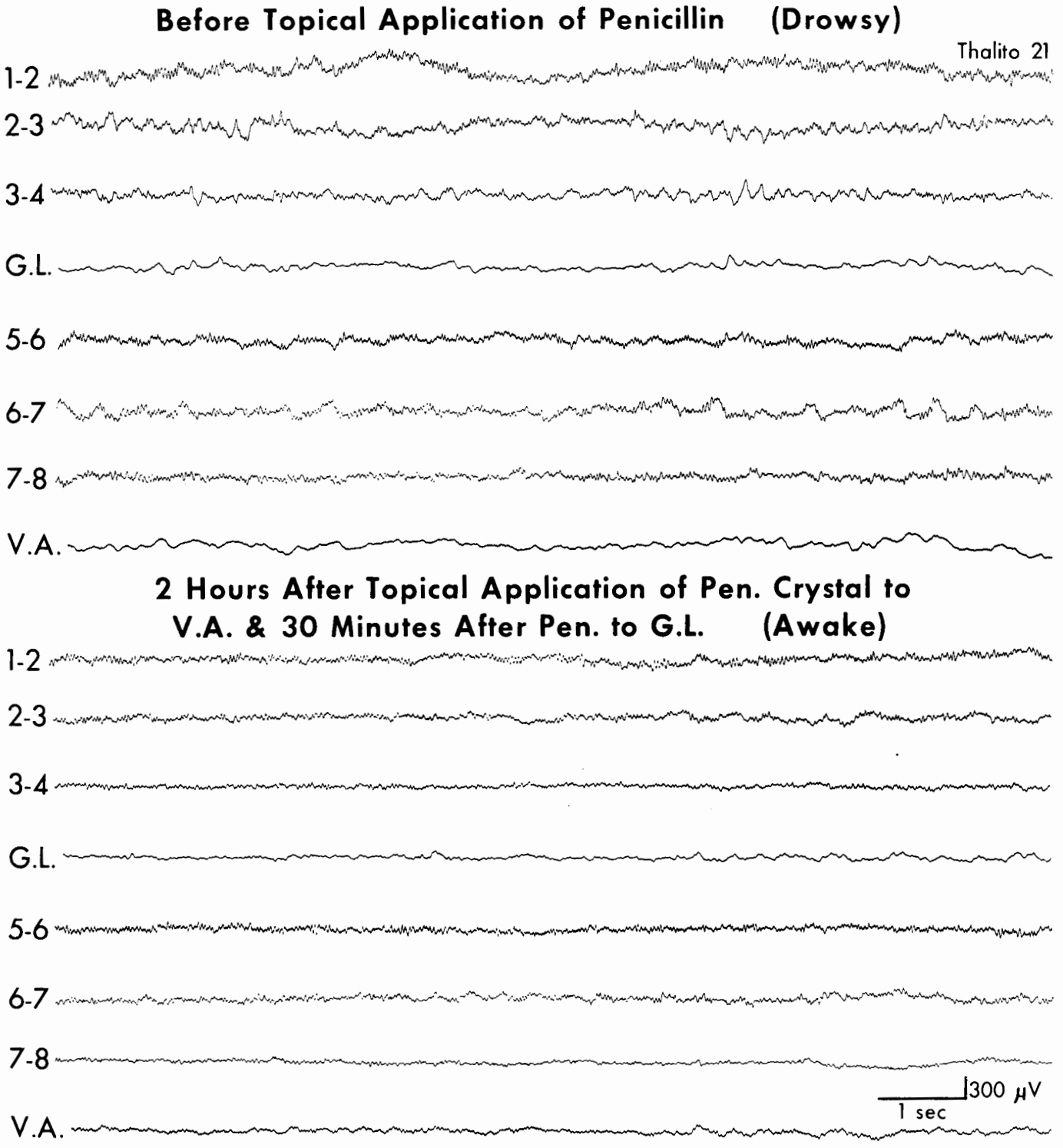

Before Topical Application of Penicillin (Drowsy)
Thalito 21
1-2
2-3
3-4
G.L.
5-6
6-7
7-8
V.A.
2 Hours After Topical Application of Pen. Crystal to
V.A. & 30 Minutes After Pen. to G.L. (Awake)
1-2
2-3
3-4
G.L.
5-6
6-7
7-8
V.A.
300 μV
1 sec

Figure 16

Figure 17. A: Cortical and depth recording from left amygdala (anterior amygdaloid area: Aa) 14 minutes after application of a penicillin crystal to Aa: large spikes appear in record from Aa.

B: The same recording 6 minutes after application of an additional penicillin crystal to the cortex near electrode 3: independent spike activity at electrode 3 in the cortex and in Aa.

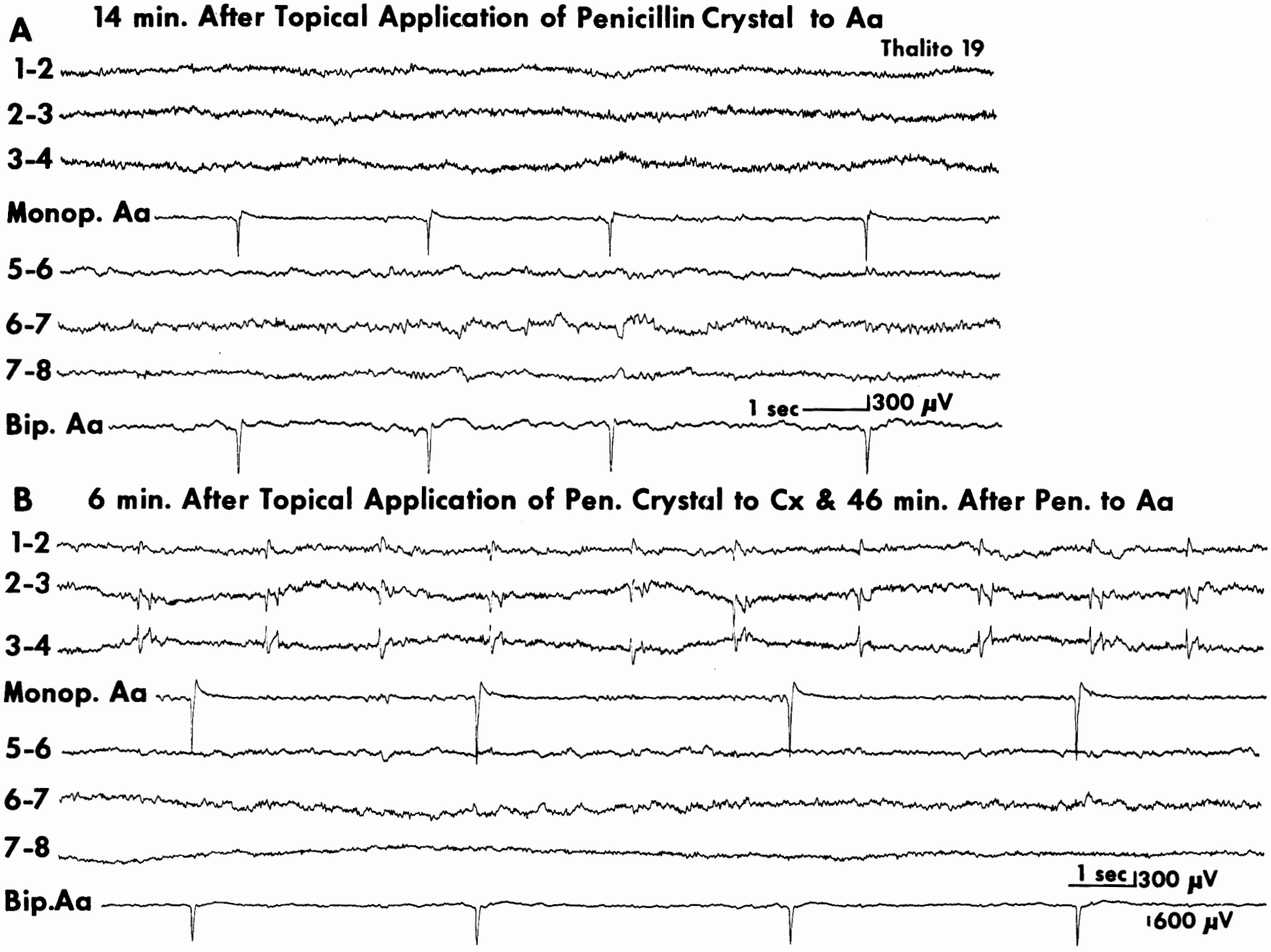

A
14 min. After Topical Application of Penicillin Crystal to Aa
Thalito 19
1-2
2-3
3-4
Monop. Aa
5-6
6-7
7-8
1 sec
300 μV
Bip. Aa
B
6 min. After Topical Application of Pen. Crystal to Cx & 46 min. After Pen. to Aa
1-2
2-3
3-4
Monop. Aa
5-6
6-7
7-8
1 sec
300 μV
Bip.Aa
600 μV

Figure 17

SECTION III

CORRELATION BETWEEN ELECTROPHYSIOLOGICAL DATA AND DISTRIBUTION OF C_{14} PENICILLIN IN THE BRAIN

INTRODUCTION

The experiments described in sections I and II did not answer the question of how penicillin was distributed within the brain after intramuscular, diffuse cortical and topical subcortical application of the drug. It was particularly important to know whether with diffuse cortical application penicillin remained essentially confined to the cortex before concluding that epileptic alteration of neuronal behavior was really confined to the cortex in feline generalized penicillin epilepsy. This led us to study the distribution of radioactively labelled penicillin (C_{14} penicillin G) in different brain structures at the onset of the generalized epileptic discharges induced by intramuscular injection or by diffuse cortical application of a penicillin solution containing radioactive C_{14} penicillin. We also studied the distribution of the radioactive drug following its topical administration to different subcortical structures. The goal of this study was to correlate the electroencephalographic findings recorded from the cerebral cortex and from subcortical structures with the distribution of C_{14} penicillin at these two levels.

MATERIALS AND METHODS

Twenty-five cats of both sexes weighing between 2.6 - 3.9 kg underwent a tracheostomy under ether anaesthesia. Ventilation was then maintained with an intermittent positive pressure Bird Mark 14 respirator

and halothane at a concentration of 2 - 2½% was given as an anesthetic agent. A wide bilateral craniectomy was performed preserving the dura mater intact, or removing it in some animals. Intracerebral electrodes were implanted in different thalamic and extrathalamic subcortical structures using the coordinates of Jasper and Ajmone Marsan's (1954) stereotaxic atlas of the cat diencephalon.

The intracerebral electrodes consisted of bipolar concentric 24 gauge stainless steel needles with an interelectrode distance of 0.1 mm and a resistance of 40 KΩ. Following electrode implantation, the animals were painlessly fixed in a Kopf semichronic head holder without eye or ear bars. At this point halothane administration was discontinued and the animals received periodic injections of small amounts of gallamine triethiodide (Flaxedil, 4 mg/cc) and of fentanyl citrate (Sublimaze 0.015 mg/ml) a potent narcotic analgesic, intravenously. The expired CO_2 was monitored with a Beckman infrared analyzer. The respiratory rate was adjusted to maintain the CO_2 level at about 4%. The surface EEG was recorded with silverball electrodes applied against the dura overlying the sigmoid gyri and middle suprasylvian bilaterally (Fig. 1B).

Bipolar and monopolar EEGs were recorded from the surface and depth electrodes on an 8-channel Elema-Schönander Mingograf machine. Single shock and low frequency (3-10 cps) repetitive stimulation was delivered through the intracerebral electrodes using a constant current Nuclear Chicago stimulator, in order to test for the position of the electrodes by using electrophysiological criteria.

Following stimulation of the nonspecific thalamic nuclei, spindle activity or recruiting responses were elicited from both hemispheres. Stimulation of specific thalamic nuclei mostly yielded evoked potentials which were confined to their ipsilateral cortical projection areas.

The penicillin solution containing labelled drug (C_{14} penicillin) was administered through three different routes: (1) it was injected intramuscularly, (ii) it was applied diffusely and bilaterally to the cortex in a weak solution (100 I.U/hemisphere) and (iii) it was applied topically by microinjection to subcortical structures. The techniques of these procedures were the same as previously described.

1) Intramuscular administration of an aqueous penicillin solution containing C_{14} penicillin: Five cats received an intramuscular injection of an aqueous penicillin G solution (350,000-400,000 I.U./kg) containing 25-50 μCi of C_{14} penicillin G. A continuous EEG recording obtained from cortical and subcortical structures was started at the time of penicillin administration. The subcortical electrical activity was recorded from nonspecific thalamic nuclei (oral pole of N. reticularis, N. centralis medialis, N. ventralis anterior and N. paracentralis), specific thalamic nuclei (medial geniculate body, lateral geniculate body and N. lateralis posterior) and from the caudate nucleus. The animals were killed under pentobarbital anaesthesia (Nembutal 25-30 mg/kg intraperitoneally) at or shortly after the time of onset of the generalized penicillin epileptic discharges (approximately 1½ - 2 hours after intramuscular penicillin injection). Samples of plasma, CSF and brain tissue for radioactive assay were obtained using techniques to be described later.

2) Diffuse bilateral cortical application of an aqueous penicillin solution containing C_{14} penicillin: In 3 animals, an aqueous penicillin G solution (100 I.U./hemisphere) containing 1 μCi of C_{14} was applied bilaterally to widespread cortical areas. This procedure was the same as described previously. Bipolar EEG recordings were obtained from the cortex and from nonspecific thalamic nuclei (N.C.M. and V.A.). The animals were killed shortly after the onset of the generalized cortical epileptic activity. In these experiments the latency of onset of epileptic activity varied from 8 minutes to 1 hour. Plasma, CSF and brain samples were obtained and prepared for radioactive measurement.

3) Topical application of an aqueous penicillin G solution containing labelled drug (C_{14} penicillin) to subcortical structures: In 11 animals, a penicillin G solution (750-2,000 I.U.) containing 0.05-0.2 μCi of C_{14} penicillin was applied topically to subcortical structures (nucleus centralis medialis, nucleus centralis lateralis and caudate nucleus). A stereotaxically driven Hamilton microsyringe (10 μl capacity) was used for the microinjection of the drug. The total volume of the solution injected varied from 1.5 to 3.5 μl. The EEG was recorded from the cortex and from those subcortical structures to which penicillin had been applied. Both the electrode implantation and the microinjection techniques were the same techniques as previously reported by us. One to two hours following the topical application of penicillin to subcortical structures, the animals were killed and samples of plasma, CSF and of several brain structures were obtained for radioactive analysis.

In all our animals, the plasma and CSF samples were obtained while the cats were alive and under pentobarbital anaesthesia. After thoracotomy, a blood sample was obtained puncturing the left ventricle of the heart. Then, 1 cc of CSF was drained from the cisterna magna using a tuberculine syringe. These two samples were taken at an interval of 2 minutes. Then the right atrium was sectioned and the animals were exsanguinated. Following the animal's death, the dura mater was dissected and the pia mater was removed with the aid of a surgical microscope and microsurgery instruments. With the brain still in situ, samples of cortical grey matter were obtained after careful isolation from the white matter. The brain was then removed from the skull and samples of thalamus, caudate nucleus and subcortical white matter (internal capsule) were obtained. In animals which received intramuscular or bilateral diffuse cortical application of penicillin, the cortical and subcortical brain samples were obtained from either side of the brain. In animals which received a thalamic microinjection of penicillin, cortical grey matter samples were taken from the ipsilateral as well as from the contralateral hemisphere. Samples of caudate nucleus and of internal capsule were obtained from the side contralateral to the injection, except in one case in which the penicillin had been injected into the caudate nucleus. In all animals, the thalamic sample included the midline thalamus. The brain samples were weighed in an analytic balance (Mettler H51). We attempted to remove samples of the same weight in all our experiments (approximately 130-150 mg). Following liquefaction of

the brain samples with Soluene 350 (2 cc), Instagel (15 cc) was added to the plasma, CSF and brain samples. In each sample, a radioactivity count was taken for 8-10 minutes using a SL 32 liquid scintillation spectrometer. A quenching curve was established by adding increasing concentrations of chloroform to an originally pure sample of C_{14} penicillin. Using this curve we were able to convert c.p.m. (counts per minute) values into d.p.m. (disintegration per minute) values, according to the channels ratio method. Knowing the amount of radioactive penicillin contained in the penicillin solution injected or applied, the d.p.m. count detected in our samples of brain tissue, plasma and CSF could be expressed in terms of I.U. penicillin/ml of plasma, or CSF. This method allowed us to study the differential distribution of penicillin in brain, plasma and CSF.

Samples of plasma, CSF and brain tissue obtained from an animal which did not receive C_{14} penicillin were processed in the scintillation counter in the same manner for 10 minutes. The results obtained from this animal determined the background level of radioactivity in the absence of C_{14} penicillin. This value was subtracted from every reading carried out in samples obtained from animals which had received C_{14} penicillin.

It was conceivable that the total radioactive count measured in our samples of brain tissue in those animals which had received C_{14} penicillin intramuscularly could be partially accounted for by the C_{14} penicillin remaining in the cerebral blood vessels after exsanguination and hence

not entirely represent penicillin taken up by brain tissue. To determine whether this was a significant complicating factor in our experiments, 5 animals underwent a craniectomy under pentobarbital anaesthesia (Nembutal 30 mg/kg intraperitoneally). RISA, a radioactive compound which does not significantly diffuse into extravascular tissue and remains confined to blood vessels, was injected intravenously in these animals (50 µCi i.v. per animal). Fifteen to 20 minutes after RISA administration, the same procedures as already described were carried out in these animals in order to obtain plasma, CSF and brain tissue samples which were counted in a Well Gamma Counter. The level of radioactivity was expressed in d.p.m./g of brain tissue or in d.p.m./cc of CSF or plasma (d.p.m. = disintegration per minute).

Results

1) Effects of intramuscular administration of penicillin (350,000-400,000 I.U./kg) containing C_{14} penicillin (25-50 µCi):

Following intramuscular injection of this amount of penicillin, generalized and bilaterally synchronous bursts of epileptic activity were recorded from the cerebral cortex as was described previously. The epileptic activity initially consisted of bursts of spike and wave or multiple spike and wave activity at a frequency varying from 3-4.5 cps with a burst duration ranging from 2-5 seconds. Subcortical structures also

showed synchronous paroxysmal discharges in these animals (Fig. 18). The abnormal electrical activity recorded from subcortical structures was variable. Although discharges resembling spike and wave complexes could be observed in various subcortical structures, they were most often recorded from the nonspecific thalamic nuclei (nucleus centralis medialis, nucleus reticularis, nucleus ventralis anterior, nucleus paracentralis). The bursts recorded from the specific thalamic nuclei or extrathalamic sites consisted mainly of slow sharp and slow wave complexes or of slow wave activity (Fig. 18). The onset of the epileptic activity occurred 1-2 hours after the penicillin administration ($\bar{X}$ = 110 minutes). Samples of plasma, CSF and brain tissue for radioactive counting were obtained shortly after the onset of the generalized epileptic activity ($\bar{X}$ = 150 minutes).

Figure 19 and Table V A shows the mean value and the standard error of the mean (SEM) of the concentration of penicillin in different brain structures, plasma and CSF, in the cats in which penicillin had been injected intramuscularly.

Penicillin was widely distributed in cortical and subcortical structures. Its concentration was not significantly different in cortical than in subcortical grey matter ($p > 0.05$). The concentration of penicillin in cortical and subcortical grey matter was higher than its concentration in subcortical white matter (internal capsule) and in CSF. These findings correlate well with the EEG findings showing epileptic bursts

in the cortex with simulataneous paroxysmal activity appearing in some subcortical structures (Fig. 18). The highest concentration of penicillin was detected in plasma and the lowest concentration of penicillin was seen in the CSF. This indicates the existence of an actively functioning blood brain barrier in these animals, which prevents a free passage of penicillin from the plasma to the CSF compartment.

2) Effects of diffuse bilateral cortical application of a weak penicillin solution (100 I.U./hemisphere) containing C_{14} penicillin (1 μCi): Generalized and bilaterally synchronous bursts of epileptic activity were recorded only from the cortex and not from subcortical structures following diffuse administration of a weak penicillin solution to the cortex of both hemispheres (Fig. 20). The pattern of the epileptic activity recorded from the cortex was very similar to the epileptic activity recorded from the cortex in animals which had received high doses of penicillin intramuscularly as had been shown previously.

In some animals, irregular and low amplitude slow wave activity was recorded from subcortical sites during the cortical epileptic bursts. However, this subcortical slow wave activity appeared always later than did the cortical epileptic bursts (Fig. 20). In these animals the concentration of penicillin was significantly higher ($p < 0.02$) in the cortical grey matter than in subcortical grey or white matter, plasma and CSF (Fig. 21 and Table VB). Again, there is good correlation between the penicillin distribution and the EEG findings since epileptic activity was recorded from the cortex only.

3) Effects of topical application of a penicillin solution (750-2,000 I.U.) containing C_{14} penicillin (0.05-0.2 μCi) to subcortical structures: In 6 animals, no epileptic activity was ever recorded neither from the cortex nor from thalamic or extrathalamic structures to which penicillin was applied (N. centralis medialis in 5 animals, caudate nucleus in one animal). However, two other interesting features were observed. Following topical application of penicillin to nonspecific thalamic nuclei (N.C.M.) intermittent bursts of rhythmic slow waves were recorded from this area (Fig. 22). Very little activity of this kind had been seen before topical administration of penicillin. Furthermore the cortical background EEG activity was also changed after thalamic application of penicillin. Prior to thalamic administration, frequent bursts of slow waves were recorded from the cortex at times when the animal was drowsy. After thalamic administration of penicillin the cortex exhibited a rather persistent low and medium amplitude fast activity (Fig. 22). In these animals the concentration of penicillin in the thalamus was significantly higher ($p < 0.02$) than its concentration in the cortex, caudate nucleus, white matter (internal capsule) and plasma (Fig. 23 and Table VC). However, the concentration of penicillin in the CSF was almost as high as its concentration in the thalamus. A possible explanation of this phenomenon will be given in the Discussion. In spite of a very high concentration of penicillin in the thalamus, no epileptic activity was recorded from this structure or from the cortex (Fig. 22). It is interesting that the concentration of penicillin in the cortex of these animals was only slightly lower (3.52 ± 0.50 IU/g,

see Table V) than the cortical concentration of the drug in those animals in which penicillin had been applied diffusely to the cortex (5.22 ± 1.32 IU/g, see Table V). The finding that in spite of the presence of penicillin in the cortex in animals with subcortical topical application of penicillin, no epileptic activity was recorded, will be discussed later.

In the 5 remaining animals, seizure discharges lasting up to 20 seconds and consisting of rhythmic sharp wave activity at a frequency of 6-8 cps were simultaneously recorded from the thalamus and cortex predominantly on the side ipsilateral to the injection (Fig. 24). Independent thalamic epileptic activity was never seen. Also, in some of these animals, high voltage sharp waves were recorded from the cortex ipsilateral to the injection site of penicillin. In none of these instances did the epileptic manifestations resemble those seen after intramuscular or diffuse cortical application of penicillin. In 4 of these animals the penicillin had been applied to the N. centralis medialis and in one to the nucleus centralis lateralis. In 2 of these animals, the epileptic activity appeared soon after a bilateral mesencephalic reticular formation lesion had been made. In a third animal, autopsy revealed a cerebellar hemorrhage involving the brain stem. In the fourth and fifth animal, no anatomical lesions possibly related to the appearance of epileptic discharges were found.

The distribution of penicillin in this group of cats (Fig. 25 and Table VD) shows that the mean thalamic and cortical concentration of penicillin was higher both in thalamus and cortex (1095.32 ± 859.10 IU/g for thalamus and 8.40 ± 1.56 IU/g for cortex, see Table V) than that found in animals with thalamic application of penicillin which did not develop epileptiform discharges (320.97 ± 156.73 IU/g for thalamus and 3.52 ± 0.50 IU/g for cortex, see Table V). However the difference between the two groups for both structures does not reach statistical significance ($p > 0.3$).

4) Ratio of RISA and penicillin concentration in brain: Since in animals in which a solution of C_{14} penicillin had been injected intramuscularly the highest concentration of the drug was found in plasma, the total radioactivity measured from different brain samples could conceivably be partially accounted for by radioactive penicillin remaining in the cerebral blood vessels after exsanguination. Since RISA is a radioactive compound which does not significantly diffuse outside the intravascular compartment, the radioactive count obtained from different brain samples after exsanguination in an animal which received RISA intravenously is necessarily produced by the blood which remained in the brain after exsanguination. Since the contamination of brain samples by RISA remaining in the intravascular compartment is largely but not exclusively dependent upon the plasma concentration of this drug, we expressed our results in brain tissue/plasma ratio of radioactivity. Similar ratios were calculated in those five animals which had received a solution of radioactive penicillin intramuscularly.

Figure 26 illustrates a comparison between the brain plasma ratios for RISA (stippled bars) and the brain plasma ratios for penicillin (black bars) in different brain structures and CSF after intramuscular injection of penicillin. It is clear from this comparison that the total radioactivity measured from different brain samples in animals which had received penicillin intramuscularly, depended mainly upon an actual uptake of penicillin by brain and was not caused by a radioactive penicillin residue left behind in cerebral blood vessels after exsanguination. The real uptake of penicillin by the different samples of brain tissue can be calculated by subtracting the radioactive count attributable to the C_{14} penicillin remaining in cerebral blood vessels. This value can be calculated from the brain/ plasma ratio of RISA. (Figure 19 presents corrected values reflecting the actual uptake of penicillin by brain.)

In animals in which the solution of C_{14} penicillin was widely applied to the cortical surface or directly injected into subcortical structures, the plasma concentration of penicillin was minimal and therefore the actual concentration of this drug in different brain structures was considered to represent a real uptake.

SUMMARY

The distribution of C_{14} penicillin in different brain structures at the time of onset of generalized epileptic discharges following intramuscular injection showed no statistically significant difference between penicillin concentration in cortical and in subcortical grey matter. In animals which underwent bilateral cortical application of penicillin the uptake of this drug by cortical grey matter was significantly higher than the uptake achieved by subcortical grey matter structures (thalamus and caudate nucleus) or by subcortical white matter (internal capsule) ($p < 0.02$). These observations militate in favour of a cortical onset of the epileptic activity characterized by generalized bilaterally syndronous spike and wave activity.

No such epileptic activity was recorded from subcortical or cortical structures following microinjection of C_{14} penicillin to the thalamus. The uptake of penicillin in these experiments was significantly higher in thalamic than in other subcortical or cortical structures ($p < 0.02$). The epileptic activity seen in some animals after thalamic injection of penicillin was of a different type and was seen in animals in which both thalamus and cortical concentration of penicillin were relatively high.

These experiments support a cortical origin of the epileptic neuronal behaviour in feline generalized penicillin epilepsy and suggest that subcortical structures such as the thalamus are insensitive to the epileptogenic action of penicillin. However, it is possible that penicillin increases the excitability of the thalamic and extra-thalamic neuronal pool.

Figure 18: Epileptic activity recorded simultaneously from the cerebral cortex and some subcortical structures (left caudate nucleus and oral pole of right nucleus reticularis of the thalamus) 1½ hours after intramuscular injection of 400,000 I.U. of penicillin.

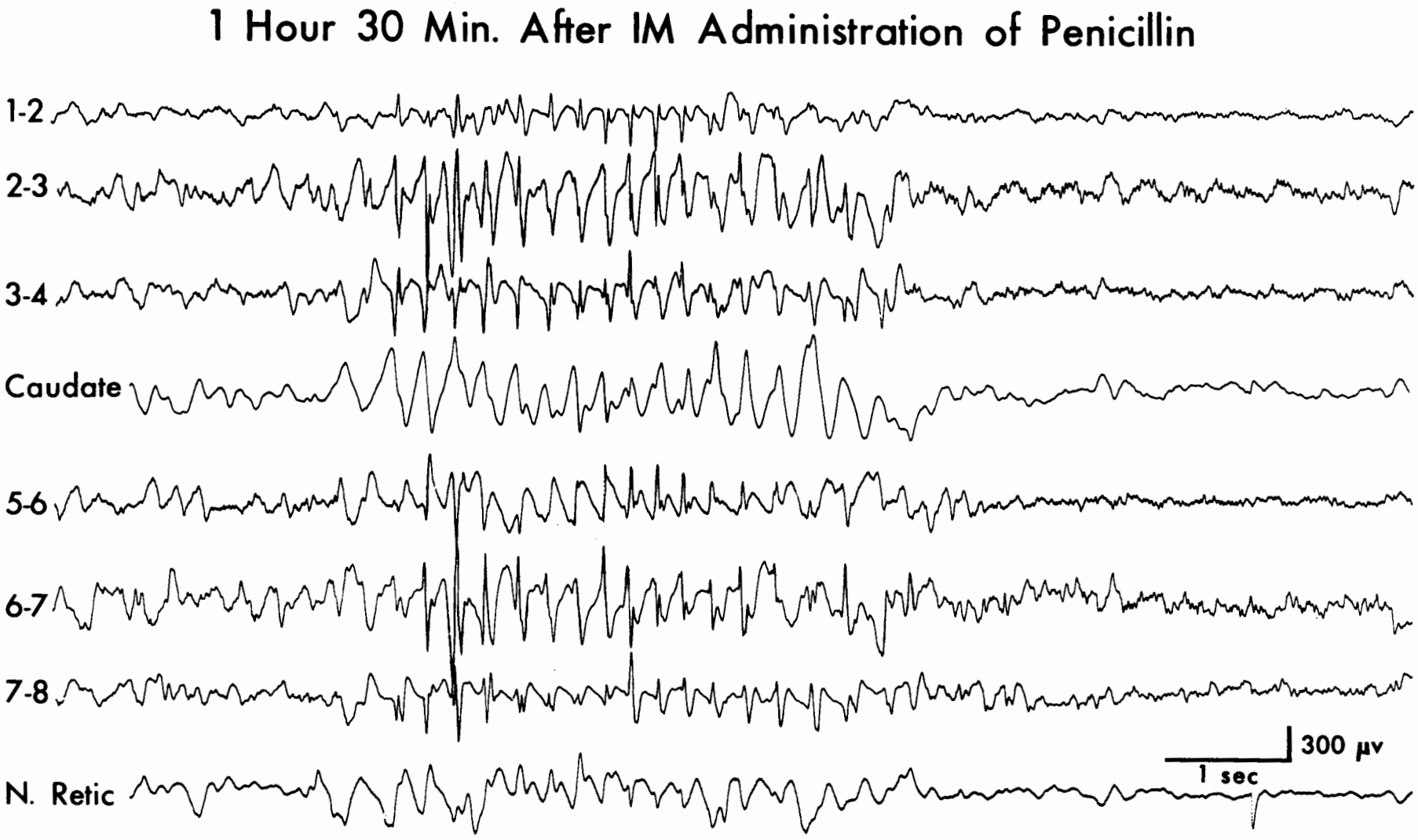

1 Hour 30 Min. After IM Administration of Penicillin
1-2
2-3
3-4
Caudate
5-6
6-7
7-8
300 μv
1 sec
N. Retic

Figure 18

Figure 19: Uptake of penicillin in brain, CSF and plasma after intramuscular administration 350,000 - 400,000 I.U. of penicillin (pooled results of 5 experiments). In this and the subsequent bar graphs the thick bars indicate the means and the small bars the standard errors of the means. (In some cases the standard error of the mean was too small to be represented in the bar graphs. For actual values, see Table V).

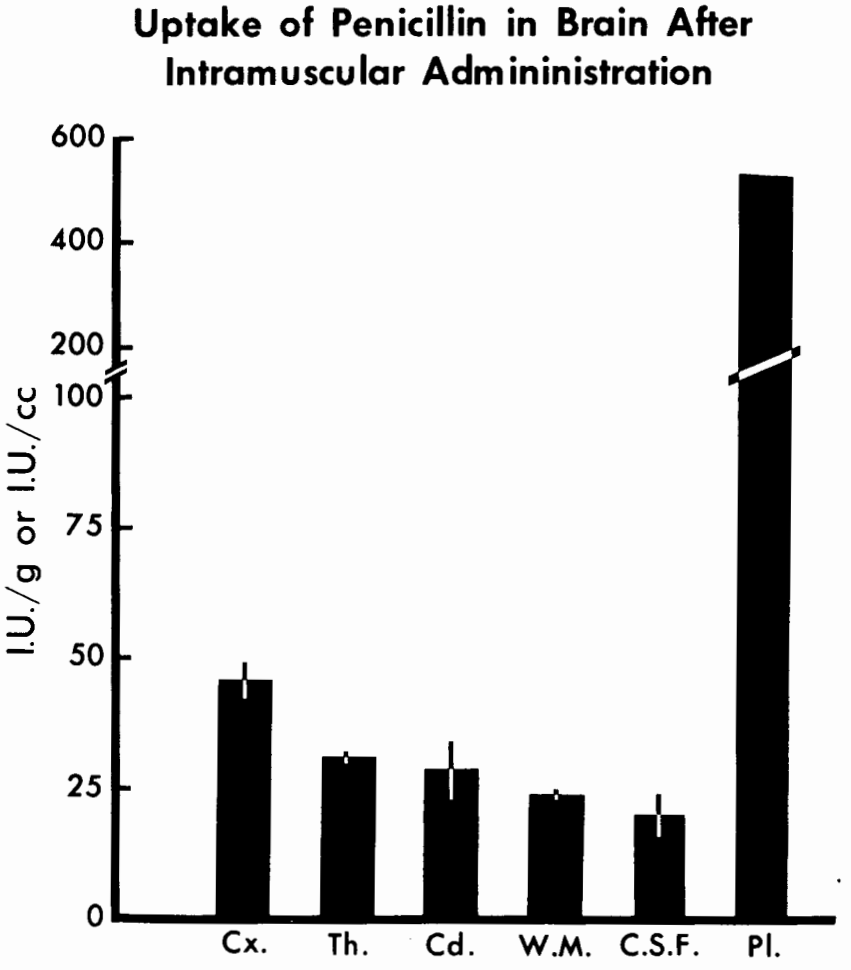

Uptake of Penicillin in Brain After
Intramuscular Admininistration
I.U./g or I.U./cc
600
400
200
100
75
50
25
0
Cx.
Th.
Cd.
W.M.
C.S.F.
Pl.

Figure 19

Figure 20: Bilaterally synchronous epileptic activity after bilateral diffuse cortical application of a weak penicillin solution (100 I.U./hemisphere). EEG recording obtained from the cortical surface and midline N.C.M.

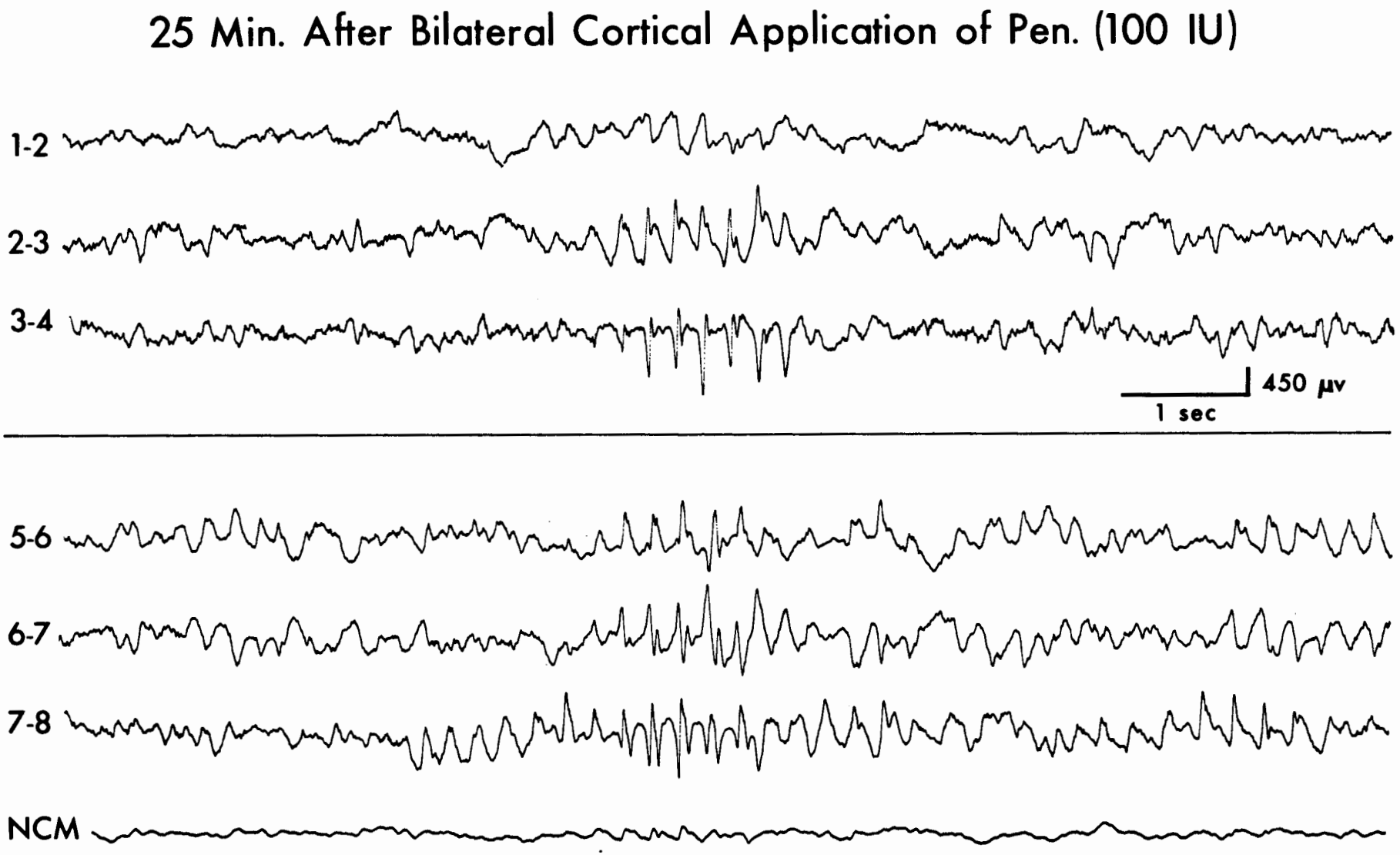

25 Min. After Bilateral Cortical Application of Pen. (100 IU)
1-2
2-3
3-4
450 µv
1 sec
5-6
6-7
7-8
NCM

Figure 20

Figure 21: Uptake of penicillin in brain, CSF and plasma after bilateral diffuse cortical application of a weak penicillin solution (100 I.U./hemisphere - pooled results of 3 experiments).

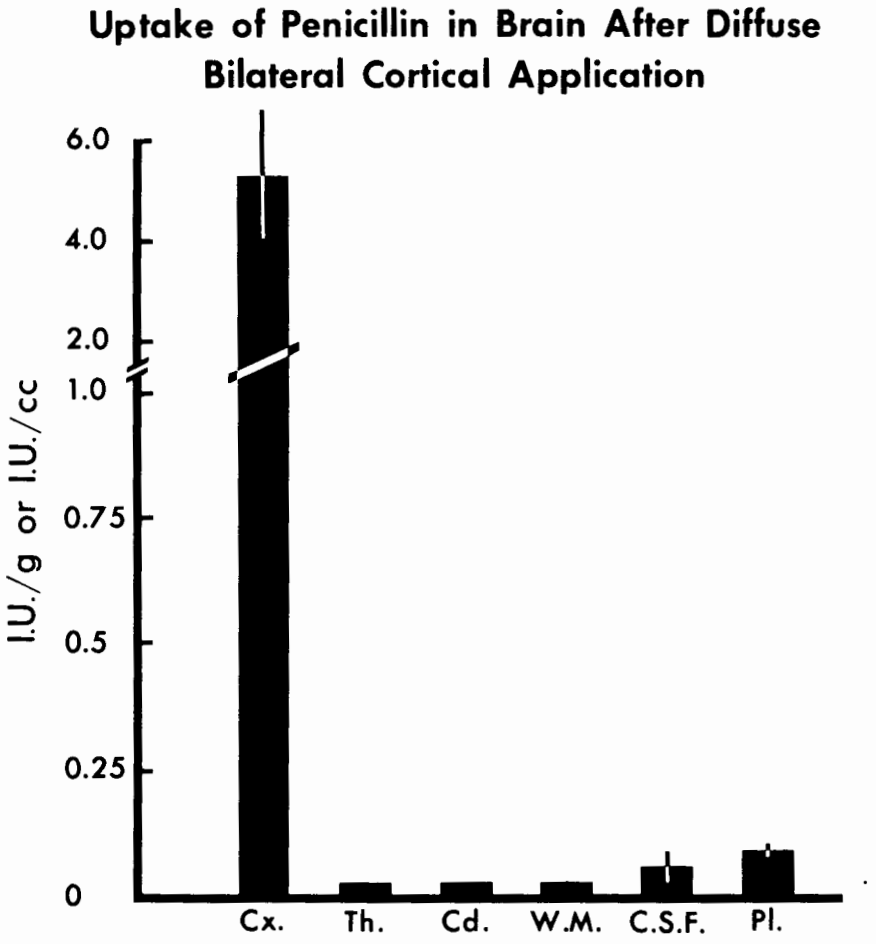

Uptake of Penicillin in Brain After Diffuse Bilateral Cortical Application
I.U./g or I.U./cc
6.0
4.0
2.0
1.0
0.75
0.5
0.25
0
Cx.
Th.
Cd.
W.M.
C.S.F.
Pl.

Figure 21

Figure 22: EEG recorded 1 hour and 25 minutes after topical application of 2,000 I.U. of penicillin to nucleus centralis medialis of the thalamus (N.C.M.). Note absence of any epileptic activity and intermittent slow wave activity in the thalamus.

1 Hour 25 Min. After Thalamic Topical Application of Penicillin (2,000 IU)

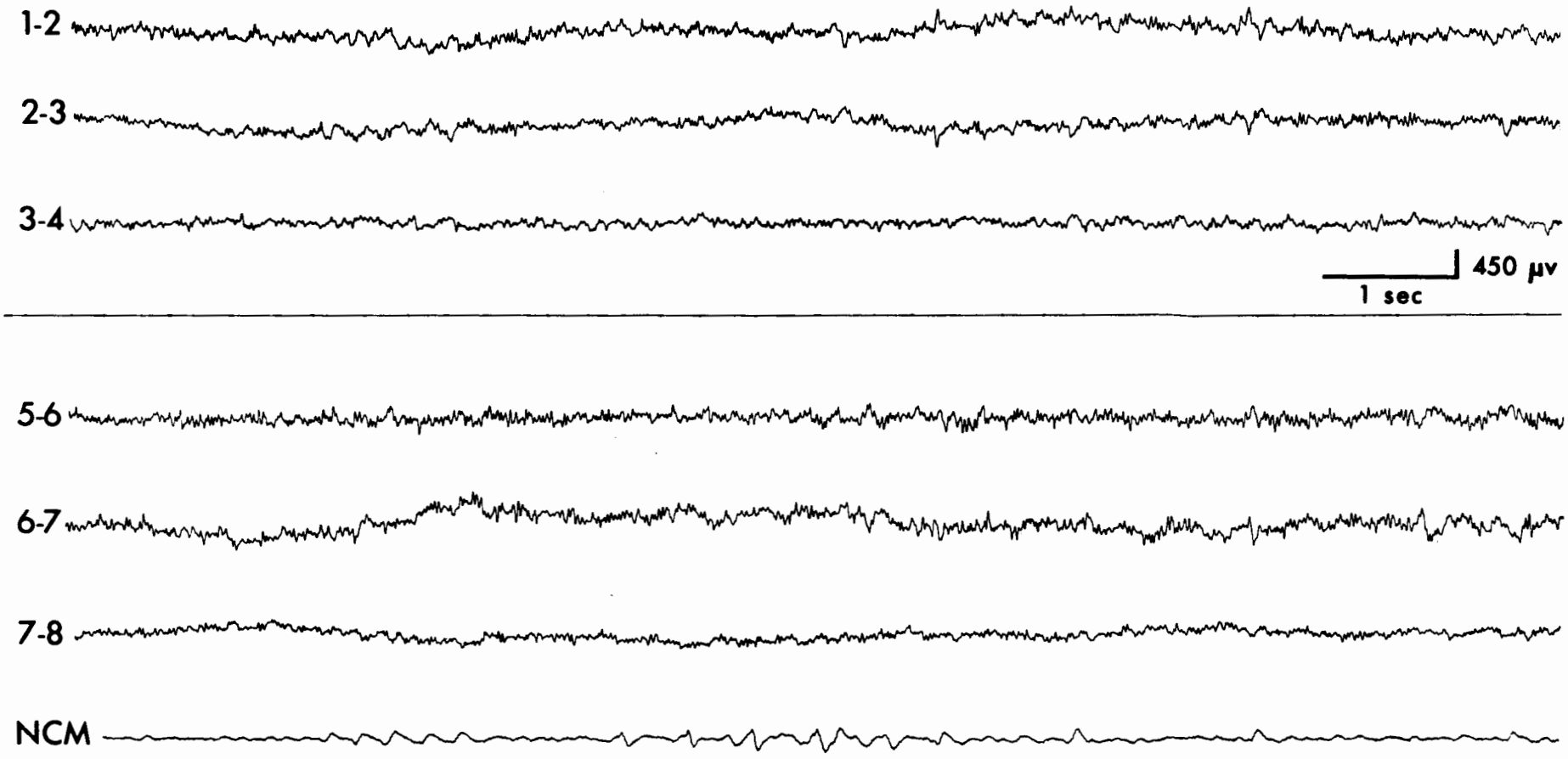


Figure 22

Figure 23: Uptake of penicillin in brain, CSF and plasma after loca thalamic application of penicillin (750-2,000 I.U.) in cats without epileptic discharges (pooled results of 6 experiments).

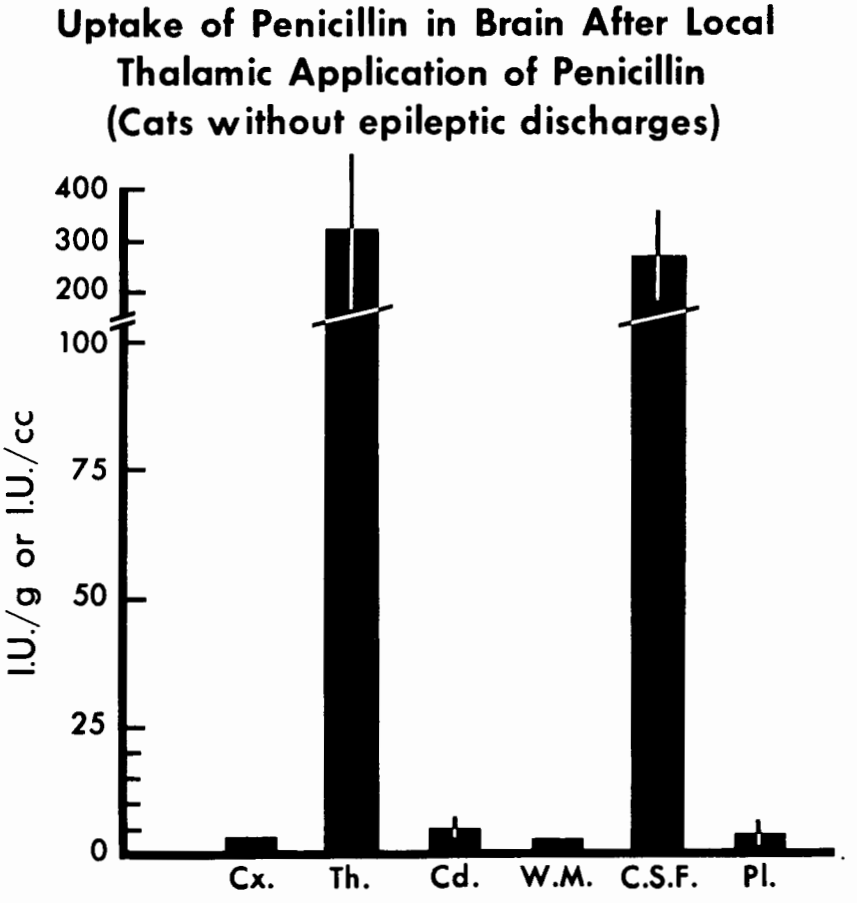

Uptake of Penicillin in Brain After Local Thalamic Application of Penicillin (Cats without epileptic discharges)
I.U./g or I.U./cc
400
300
200
100
75
50
25
0
Cx.
Th.
Cd.
W.M.
C.S.F.
Pl.

Figure 23

Figure 24: Seizure discharges recorded in cortex and thalamus after topical application of penicillin (2,000 I.U.) to nucleus centralis medialis of the thalamus (N.C.M.) B is the direct continuation of A.

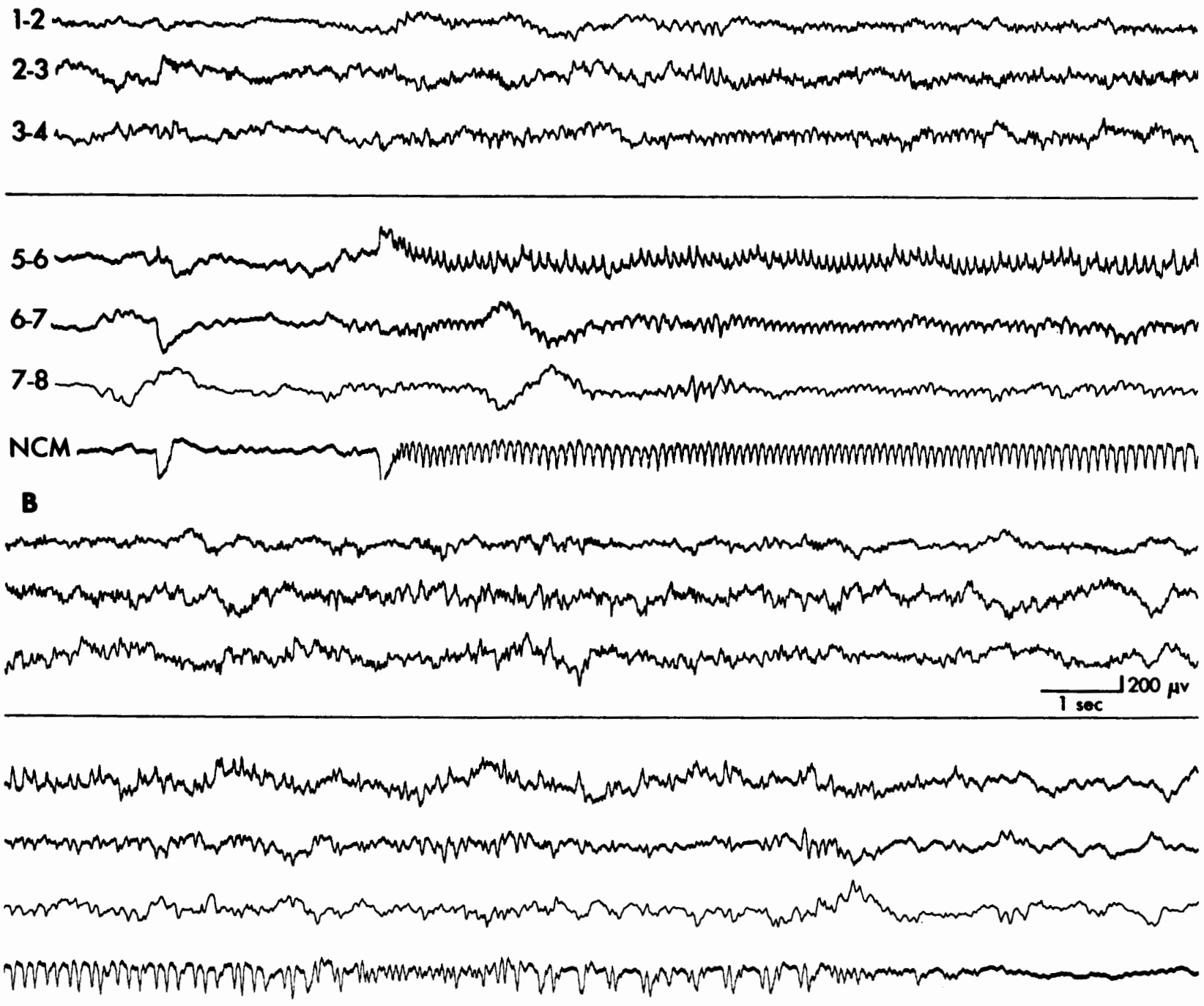

A
1 Hour 15 Min. After Thalamic Application of Penicillin - 2,000 IU
1-2
2-3
3-4
5-6
6-7
7-8
NCM
B
200 μv
1 sec

Figure 24

Figure 25: Uptake of penicillin brain, CSF and plasma after local thalamic application of penicillin (750-2,000 I.U.) in cats with epileptic discharges (pooled results of 5 experiments).

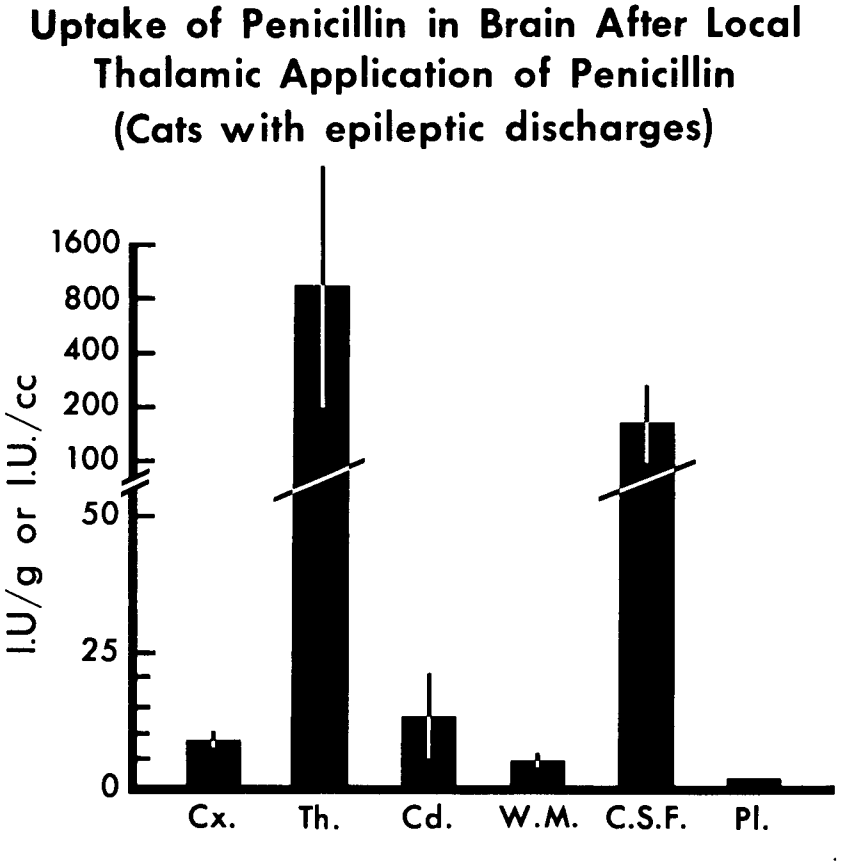

Uptake of Penicillin in Brain After Local Thalamic Application of Penicillin (Cats with epileptic discharges)
I.U/g or I.U./cc
1600
800
400
200
100
50
25
0
Cx.
Th.
Cd.
W.M.
C.S.F.
Pl.

Figure 25

Figure 26: Tissue-plasma ratios of penicillin and RISA in brain and CSF.

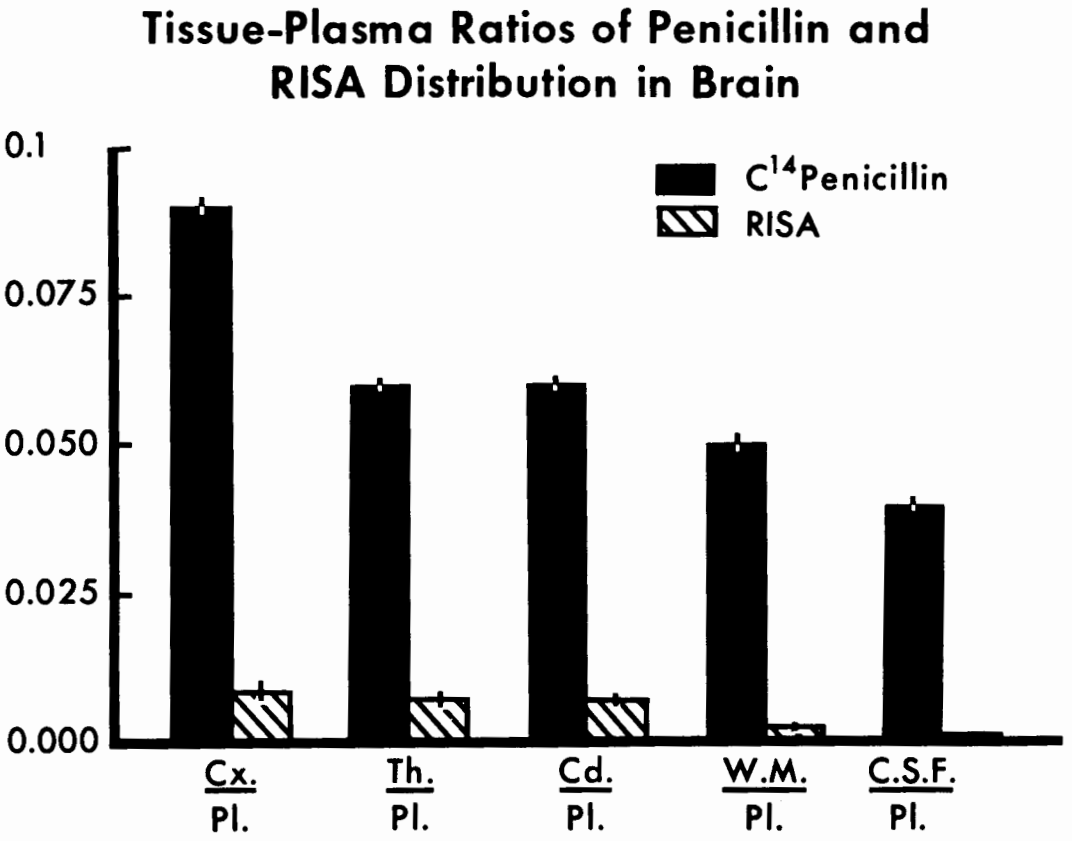

Tissue-Plasma Ratios of Penicillin and
RISA Distribution in Brain
0.1
0.075
0.050
0.025
0.000
C14Penicillin
RISA
Cx./Pl.
Th./Pl.
Cd./Pl.
W.M./Pl.
C.S.F./Pl.

Figure 26

TABLE V

(A) Uptake of Penicillin in Brain After Intramuscular Administration*

(data expressed in I.U./g or I.U./cc)

	Cx	Th	Cd	WM	CSF	Pl
Mean	45.55	30.91	28.87	23.92	19.98	537.00
SEM	3.05	0.20	5.69	1.00	3.57	0.50

(B) Uptake of Penicillin in Brain After Diffuse Bilateral Cortical Applicatio

(data expressed in I.U./g or I.U./cc)

	Cx	Th	Cd	WM	CSF	Pl
Mean	5.22	0.04	0.04	0.04	0.06	0.09
SEM	1.32	0.001	0.001	0.01	0.03	0.002

(C) Uptake of Penicillin in Brain After Local Thalamic Application of Penicillin. (I.U./g or I.U./cc)

(Cats without epileptic discharges).

	Cx	Th	Cd	WM	CSF	Pl
Mean	3.52	320.97	5.28	2.90	272.41	4.04
SEM	0.50	156.73	1.43	0.45	78.94	2.49

(D) Uptake of Penicillin in Brain After Local Thalamic Application of Penicillin (I.U./g or I.U./cc)

(Cats with epileptic discharges).

	Cx	Th	Cd	WM	CSF	Pl
Mean	8.40	1095.32	13.03	4.55	171.91	1.59
SEM	1.56	859.10	8.03	1.51	70.73	0.29

* Real uptake of penicillin (see text re: RISA experiments).

GENERAL DISCUSSION

GENERAL DISCUSSION

Feline generalized penicillin epilepsy seems to represent the best animal model of human generalized corticoreticular (centrencephalic) epilepsy presently available. When Prince and Farrell (1969) first reported their observations on feline generalized penicillin epilepsy, they noted that epileptic activity could be recorded from both cortical and subcortical structures. It remained, however, unclear whether cortical bursts were triggered by subcortical discharges or vice versa. Gloor et al., have reported a series of experimental studies designed to clarify the interaction between cortical and subcortical structures in this model of generalized epilepsy. Their earlier studies delineated the role of the ascending reticular formation in this form of generalized epilepsy. It was demonstrated that neural activity originating in the midbrain reticular formation exerted, presumably through ascending cholinergic reticulocortical pathways, a powerful inhibitory action upon the generalized epileptiform discharges in this model (Gloor and Testa, 1974; Testa and Gloor, 1974; Guberman and Gloor, 1974). The origin of the abnormal generalized epileptiform discharges and the mechanisms precipitating their occurrence remained, however, still unclarified, although it seemed that in all probability structures above the midbrain level, presumably cortex or thalamo-cortical neural circuits were involved in their elicitation.

The present experiments carried out in animals which received penicillin intramuscularly, moved these investigations one step further and demonstrated that generalized epileptiform discharges in feline generalized penicillin epilepsy can be triggered from many parts of the cerebrum, but that the most potent trigger zones are those from which in the normal animals spindles can be elicited, particularly under barbiturate anaesthesia, or structures from which bilateral recruiting responses can be induced by low frequency stimulation. These structures comprise the midline and intralaminar nuclei of the thalamus, portions of the basal ganglia (essentially the neostriatum and the claustrum) and some posterior thalamic association nuclei (Pulvinar and L.P.). The capability of these structures to elicit such responses on appropriate stimulation has been known for many years (Jasper and Droogleever-Fortuyn, 1974; Jasper, 1949, 1960; Starzl and Magoun, 1951) and in the "classical" study of 1947, Jasper and Droogleever-Fortuyn had, of course, shown that 3 cps stimulation of the intralaminar and midline thalamic nuclei was capable in some animals of inducing 3 cps bilaterally synchronous spike and wave discharges. These discharges were, however, always phase-locked to the stimuli, never outlasted the end of stimulation and were frequently unilateral. They were thus considered by some as being of doubtful significance for the understanding of the pathophysiology of spontaneous generalized spike and wave discharge in human epilepsy. Later, however, Hunter and Jasper (1949) showed that stimulation in this area could reproduce in chronic experiments using

freely moving animals behavioral manifestations resembling absence attacks. In their original paper, Jasper and Droogleever-Fortuyn (1947) commented on the fact that the elicitation of the characteristic bilaterally synchronous 3 cps spike and wave response from midline and intralaminar thalamic stimulation was not easily reproducible, a finding which was later confirmed by Ingvar (1955). Our observations are in agreement with this, since we could obtain bilaterally synchronous spike and wave discharges in the normal animal before penicillin in only a few instances. However, even in these animals, the epileptic activity became more vigorous after penicillin application: it now outlasted the end of stimulation and was no longer phase-locked to the stimuli. The present study would thus confirm Jasper's and Droogleever-Fortuyn's (1947) conclusion that the intralaminar and midline thalamic nuclei are structures which have some kind of special affinity to the mechanisms involved in the genesis of generalized bilaterally synchronous spike and wave discharges. The evidence presented in the first section of this study is highly suggestive that after intramuscular penicillin injection thalamo-cortical volleys, which normally induce spindle activity, now frequently precipitate generalized spike and wave discharges. Whether this transformation from a spindle response to an epileptic burst is primarily dependent upon altered neuronal and/or synaptic mechanisms at the thalamic, at the cortical or at both levels still remained undetermined at this point.

One of the interesting findings in the present study, however, is that the triggering of generalized epileptiform bursts in feline generalized penicillin epilepsy can also be obtained from structures other than those likely to induce spindles or recruiting responses upon electrical simulation. The probability that electrical stimulation applied to these structures is followed by a bilateral burst of generalized epileptic activity is, however, much lower than for those parts of the brain from which spindle triggering or recruiting responses can be elicited with a high degree of probability. Yet the incidence of elicitation of generalized epileptic bursts from most of these less effective structures which are widely distributed throughout the brain and comprise thalamic, brain stem, and neocortical structures, is still well above chance level in some instances. Only occasionally in the hypothalamus, orbitofrontal cortex and amygdala does one find truly ineffective sites.

These observations indicate that the generalized discharges in this model do not depend on a single fixed pacemaker, but that almost any cerebral structure has some potential "pacemaker properties". There is no question, however, that the intralaminar and midline thalamic nuclei, the neostriatum and a few thalamic association nuclei have significantly more effective pacemaker properties than other brain structures. Whether spontaneous bursts are also primarily triggered from these structures remains unproven. However, it is likely that spike and wave

discharges represent cortical postsynaptic potentials. Since no sites were found which are more effective in precipitating generalized epileptic bursts, it is quite conceivable that spontaneous bursts are often, although not necessarily always, triggered by thalamocortical volleys originating in the "thalamic reticular system" or in structures physiologically closely associated with it (basal ganglia and some thalamic association nuclei).

In view of the often proposed importance of frontal cortex, especially its mesial part, for the initiation of generalized spike and wave discharge (Marcus and Watson, 1968; Bancaud, 1971; Bancaud et al., 1974; Goldring, 1972), it was rather surprising that the cat homologue of the frontal cortex, the gyrus proreus, was rather ineffective as a trigger area for generalized epileptic discharges. However, the cat's homologue of the primate frontal cortex is a rather insignificant structure. The large development of frontal cortex in primates may well have gone hand in hand with some reorganization of the neural connectivity of this part of the brain to other cerebral structures and thus may have led to somewhat different electrophysiological properties. It is interesting, however, that in our experiments the cingulate gyrus, among all cortical areas, seemed to be the one from which generalized epileptic bursts could be elicited with the greatest probability. This is reminiscent of the importance Lennox and Robinson (1951) ascribed to this gyrus for the genesis of epileptic seizures associated with spike and wave discharge. This observation may also have some bearing on the findings of Bancaud et al., (1974) in man which indicated that the most

effective points in the cortex from which absence seizures and/or bilaterally synchronous spike and wave activity could be elicited was the mesial frontal cortex. Many of these points included, in fact, the anterior cingulate gyrus. It should also be noted that patients with an electrographic syndrome of so-called "secondary bilateral synchrony" frequently have a mesial frontal epileptogenic focus (Tükel and Jasper, 1952).

Our observations that epileptiform or at least paroxysmal activity was also recorded from various subcortical structures at a time when generalized cortical bursts of epileptiform activity were present, is in accordance with some of the observations made in man during stereotaxic explorations of deep structures in patients presenting with bilaterally synchronous spike and wave activity in their EEGs (Williams, 1953; Rossi et al., 1968; Niedermeyer et al., 1969; Spiegel et al., 1951). In ink written conventional EEG records as in those also obtained in the present studies, the paroxysmal discharges appeared to involve cortical and subcortical structures synchronously. The question must then be asked whether the abnormal paroxysmal activity recorded at both levels originates in subcortical structures (for instance, in the thalamic nuclei) and is projected to the cortex, thus entraining abnormal activity there, or whether the subcortical activity is produced by backfiring of abnormal cortical discharge into deep structures and thus is driven by these cortical discharges. The evidence presented in animals which received

penicillin intramuscularly indicates that afferent volleys to the cortex which may originate from many parts of the brain, but particularly from the intralaminar and midline thalamus, are capable of eliciting abnormal paroxysmal activity at cortical and subcortical levels simultaneously. At first glance, this may suggest that the primary abnormality in this form of generalized epilepsy may in fact be subcortical and that the cortex responds to abnormal afferent input. However, it is also possible to assume that it is the cortex that responds abnormally to afferent impulses, whether they be normal or abnormal and that the secondary reflection of this abnormal cortical activity which is projected back to subcortical structures is what appears in the EEG records obtained from subcortical nuclei. The observation that cortical epileptiform discharges were sometimes seen before any paroxysmal abnormality appeared in subcortical recordings is somewhat in favor of this explanation.

The second series of experiments addressed itself more fully to this problem and adduced evidence suggesting that the alteration of neuronal activity responsible for the epileptic nature of the discharges in fact resides primarily in cortical grey matter. These experiments demonstrated that generalized bilaterally synchronous epileptiform discharges, often of a typical spike and wave form could be obtained after the application of a weak solution of penicillin to widespread regions of the cortex of both hemispheres. The pattern of this discharge was very similar to that obtained in animals which received high doses of penicillin intramuscularly. The main difference between the EEG

manifestations of intramuscular application of penicillin and of diffuse cortical application of penicillin is that in the former, bursts of subcortical slow waves and sometimes spike and wave-like discharges are seen synchronously with the cortical bursts, whereas in the latter, the paroxysmal discharges are virtually confined to the cortex. It seems therefore from these observations that when the epileptogenic agent responsible for the production of bilaterally synchronous spike and wave discharge in generalized feline penicillin epilepsy is applied selectively to the cortex in a widespread manner, the same cortical electrographic manifestations can be elicited as with intramuscular application. This is, however, only possible if the solution of penicillin is weak (not exceeding about 250 I.U./hemisphere). Larger concentrations induced a multiple spike pattern with generalized seizure discharges similar to those associated with generalized tonic-clonic convulsions.

With diffuse cortical application of a weak penicillin solution, not only the pattern of epileptic discharge induced was similar to that seen with intramuscular injection of penicillin, but the responses of the cortex to single shock and low frequency stimulations of various brain structures were essentially the same as those seen with such stimulations after intramuscular injection of penicillin. Again, stimulation of those structures which were highly effective in normal

animals in precipitating spindle waves or recruiting responses were found to be highly effective in producing bilaterally synchronous epileptic bursts after the cortex had been widely and bilaterally exposed to a weak, topically applied penicillin solution. Epileptic bursts could be triggered even before the onset of spontaneous bilaterally synchronous epileptiform discharge in these animals after diffuse cortical application of penicillin, as well as in 3 animals in which no spontaneous bursts occurred. Conversely, as had been the case in the study with intramuscular penicillin administration, stimulation of those structures which are ineffective in precipitating spindle waves and recruiting responses in the normal animal, showed a very low effectiveness in precipitating epileptic discharges in the animal in which the cortex of both hemispheres had been exposed widely to a weak penicillin solution. The only difference between the two sets of experiments was that caudate stimulation in the present series, although highly effective in producing spindle bursts, was not very effective in producing spike and wave activity, suggesting that perhaps some change in the excitability of subcortical structures with systemic application facilitates spike and wave discharge induced by caudate stimulation.

Since in these experiments penicillin was applied to the cortex only, and since experiments with C_{14} penicillin application to the cortex showed that diffusion of penicillin to deep brain structures was minimal, it appears likely that the epileptogenic alteration of neuronal behavior in generalized feline penicillin epilepsy resides in the cortex and not in subcortical structures. This conclusion is strengthened by the observation that topical application of penicillin to subcortical

structures, particularly to the thalamus, was completely incapable of producing any form of epileptic discharge provided there was no evidence of probable diffusion of penicillin to the cortex as had been the case in some of the C_{14} penicillin experiments in which the drug had been applied subcortically. Epileptic discharges even failed to appear after thalamic penicillin application when the drug was applied topically to the sites which had proved most effective in precipitating bilaterally synchronous spike and wave discharges in animals after intramuscular injection or after diffuse cortical application of penicillin. Some mild electrographic alterations were seen after such thalamic penicillin applications, but they were obviously insufficient for the precipitation of generalized epileptic discharge. These observations also confirm the earlier findings of Gloor et al., (1966) and demonstrate that the presence of anaesthesia in those earlier experiments had not been a factor preventing the epileptogenic action of penicillin from being exerted at the thalamic level.

The experimental findings derived from the administration of C_{14} penicillin indicated a good correlation between the presence of significant amounts of penicillin in the cortex and the presence of bilaterally synchronous generalized epileptiform discharge of the spike and wave type. This suggests a cortical location of the crucial alteration of epileptic neuronal behavior responsible for this type of epileptic discharge.

The distribution of C_{14} penicillin in different brain structures after intramuscular administration showed a slightly higher uptake of this drug in the cortical grey matter than in subcortical grey (thalamus and caudate nucleus) or white matter (internal capsule) structures, but the differences in concentration between cortical and subcortical grey matter were not statistically significant. Following intramuscular administration of penicillin there was a significant uptake of this drug by subcortical structures. Generalized epileptic bursts were recorded in these experiments from cortical and subcortical structures simultaneously. However, we know from previous studies (Gloor et al., 1966) and from our own results that subcortical structures most likely are insensitive to the epileptogenic action of penicillin.

The experiments carried out in cats which received bilateral diffuse cortical application of C_{14} penicillin suggested very strongly that the generalized epileptic activity in this model depends upon an "epileptogenic" alteration of function of cortical neurons. In these animals, epileptic bursts very similar to those observed after intramuscular administration of penicillin were recorded only from the cortex and no paroxysmal activity was seen in subcortical structures. The distribution of penicillin in these animals showed a significantly higher uptake of this drug by the cortical grey matter ($p < 0.02$) and only traces of penicillin were detected in subcortical grey matter (thalamus and caudate). It is interesting to note at this point that the cortical concentration of penicillin required to produce generalized and bilaterally synchronous

epileptic bursts recorded from the cortex (5 I.U./gm) was significantly lower than the cortical concentration of penicillin achieved following intramuscular administration of this drug (46 I.U./gm). This finding indicates a high sensitivity of cortical grey matter to the epileptogenic action of penicillin. The absence of epileptic activity in subcortical structures which only showed a small uptake of penicillin following bilateral cortical application of this drug, indicates that the presence of paroxysmal activity in subcortical structures after intramuscular injection is in some way related to the concentration of penicillin in thalamic or extrathalamic subcortical sites. This statement is not really in conflict with the observation that subcortical structures are refractory to the epileptogenic action of penicillin, but this drug could very well produce changes in the excitability of thalamic or extrathalamic neurons rendering them more responsive to the excitatory influence of corticofugal volleys. Recent data (Raines and Dretchen, 1975) have suggested that penicillin does in fact increase the excitability of simple, even nonsynaptic neural tissues. We tentatively propose that the presence of abnormal EEG activity recorded from subcortical structures during cortical epileptic bursts is probably due to an increase of excitability of these sites induced by the penicillin in such a way that corticothalamic volleys find a more receptive neuronal pool. Whatever the mechanism of this abnormal paroxysmal thalamic activity, it is clear from our experimental results following diffuse bilateral cortical application of penicillin, that this activity is not essential for the generation of cortical epileptic bursts.

The results obtained in animals in which penicillin was applied topically to the thalamus and caudate nucleus, indicated that these subcortical structures are refractory to the epileptogenic action of penicillin. No independent epileptic activity was ever recorded from subcortical sites in these experiments even in those animals which exhibited epileptiform bursts simultaneously recorded from thalamic and cortical structures. Furthermore, in most of the latter animals, clear-cut focal cortical epileptic discharges were recorded from the hemisphere ipsilateral to the injection site. We should also mention that the epileptiform bursts recorded from the thalamus consisted mainly of rhythmic sharp wave activity and no typical spike and wave activity was ever seen. Finally, the cortical distribution of C_{14} penicillin in these animals showed a high concentration of this drug in those areas from which focal or lateralized epileptic activity was recorded, which suggests that somehow some of the injected penicillin had found its way to the cortex.

In those animals which did not exhibit thalamic or cortical epileptic discharges following subcortical administration of penicillin, the distribution of this drug in different brain structures was extremely high in the thalamus and low in the cortical mantle ($p < 0.02$). Comparatively, the thalamic penicillin uptake in these experiments was almost eight times higher than the thalamic uptake of this drug in animals which received penicillin intramuscularly and which showed thalamic paroxysmal discharges.

If our tentative proposal that penicillin can increase the excitability of thalamic neurons is right, we should then expect some electrographic changes from the thalamic or cortical recordings in the presence of such a high concentration of penicillin in the thalamus. Frequent recurrent bursts of slow wave activity were recorded from the thalamus in these animals while the cortical recordings exhibited an EEG pattern resembling a state of arousal (persistent low and medium voltage fast activity). These findings were only obtained after topical application of penicillin to the thalamus. A similar arousal pattern has been recorded from the cortex following high frequency stimulation of the nonspecific thalamic nuclei by Weinberger (1965). Therefore it seems logical to propose that thalamic neurons in a state of higher excitability induced by penicillin could be responsible for the arousal EEG pattern observed in the cortex. The recurrent bursts of slow wave activity recorded from the thalamus could be an electrographic sign indicating hyperexcitable thalamic neurons and this observation could be related to the paroxysmal slow wave activity or ill defined spike and wave discharges recorded from thalamic structures during cortical epileptic bursts seen after intramuscular injection of penicillin.

The distribution of penicillin in different brain structures following topical application of penicillin to the thalamus, clearly shows a contamination of the CSF and of the cortex with penicillin. This contamination could be the result of seepage of penicillin through

the needle tract, rupture of the thalamic cavity containing penicillin into the IIIrd ventricle, dissemination of penicillin through CSF spaces while the microsyringe is introduced or could be in part due to a diffusion of penicillin across the ependymal epithelium to the CSF.

In those animals which did not exhibit epileptic activity either from thalamic or cortical structures following subcortical administration of penicillin, a moderate uptake of the drug was seen in the cortical grey matter. In spite of this fact no epileptic activity was recorded from the cortex. Two hypotheses may explain this phenomenon. Firstly, the concentration of penicillin obtained in the cortical grey matter could have been below threshold for the induction of cortical epileptic changes. Secondly, because an EEG arousal pattern was recorded from the cortex following thalamic administration of penicillin, this could have prevented the appearance of cortical epileptic discharges. It is well known that arousal induced by sensory or pharmacological stimulation, or by high frequency stimulation of the mesencephalic reticular formation (Gloor and Testa, 1974; Testa and Gloor, 1974; Guberman and Gloor, 1974) blocks or significantly reduces the epileptic activity in feline generalized penicillin epilepsy.

Finally, a brief comment regarding the ratio of RISA and penicillin distribution in different brain structures should be made: It is clear from these results that there is a significant uptake of penicillin by brain structures following intramuscular administration of this drug. In no way could the radioactivity detected in the brain be exclusively due to penicillin which remained in the intravascular compartment after exsanguination.

The experimental observations reported in animals which underwent intramuscular, bilateral cortical and thalamic administration of penicillin permit us to propose a pathophysiological mechanism for the genesis of generalized bilaterally synchronous spike and wave discharge in generalized penicillin epilepsy of the cat, which, by extrapolation, may be applicable to human generalized corticoreticular ("centrencephalic") epilepsy. According to the results of our study, it seems that widespread epileptic discharge in these conditions is dependent upon a diffuse mild epileptogenic state of the cortex. The nature of this epileptogenic state, of course, remains to be defined in precise neurophysiological terms. It is, however, apparent from our experiments that the most potent trigger mechanism for precipitating bilaterally synchronous spike and wave discharge resides in systems of the brain located subcortically which in the normal animal are involved in spindle genesis and recruiting responses. These structures are mainly centered upon intralaminar and midline nuclei of the thalamus and constitute the so-called "thalamic reticular system" (Jasper, 1949; Jasper and Ajmone Marsan, 1954). Some association nuclei such as the pulvinar and the nucleus lateralis posterior are also fairly effective trigger zones as well as some structures belonging to the basal ganglia, essentially the neostriatum.

These experiments thus reaffirm the importance of the thalamic reticular system in the pathogenetic mechanism of generalized bilaterally

synchronous spike and wave discharge as originally proposed by Jasper and Droogleever-Fortuyn (1947). By thus ascribing important and distinctive roles to cortical and subcortical structures in the physiopathogenesis of this kind of seizure discharge, our experimental findings and the conclusions derived therefrom may, it is hoped, lay to rest the old controversy between the "centrencephalic" as opposed to the "cortical" hypothesis of generalized seizures associated with bilaterally synchronous spike and wave discharge. These considerations lend support and define more precisely the "corticoreticular" hypothesis proposed earlier by Gloor (1968, 1969).

GENERAL SUMMARY AND CONCLUSIONS

Feline generalized penicillin epilepsy seems to represent the best animal model of human generalized corticoreticular (centrencephalic) epilepsy presently available. The role of cortical and subcortical structures in this form of epilepsy was studied in acute and chronic experiments in cats with implanted skull and intracerebral electrodes. Single shock and low frequency repetitive stimulation of subcortical sites from which prior to penicillin administration spindle activity and recruiting responses could be elicited, readily triggered epileptiform discharges in the same animals after penicillin. These structures comprised the intralaminar and midline thalamic nuclei, the neostriatum, and some posterior thalamic association nuclei (pulvinar and nucleus lateralis posterior). Subcortical and cortical structures which prior to penicillin elicited neither spindle activity nor recruiting responses were significantly less effective in triggering generalized epileptic bursts after penicillin injection. The probability with which such bursts were elicited from these structures was still, however, definitely above chance level in most instances.

It is concluded that in feline generalized penicillin epilepsy, a large number of potential "pacemaker" structures exist in the brain, but that activity in subcortical nuclei involved in spindle generation and recruiting responses have the highest potential of initiating generalized epileptic discharges of the spike and wave type.

Topical applications of a weak penicillin solution to large areas of cortex of both hemispheres (50-250 I.U./hemisphere) in the cat, induces bilaterally synchronous epileptiform discharges, often of a characteristic spike and wave type, which in all respects are similar to those obtained with intramuscular injection of high doses of penicillin. The only difference is that with diffuse cortical application no synchronous paroxysmal high voltage discharges are seen in subcortical structures. This cortically induced generalized epileptiform condition responds in the same manner as that induced by intramuscular injection of penicillin to single shock and low frequency stimulation of a variety of brain structures: bilaterally synchronous epileptiform discharges are precipitated with a high probability by stimulations in those subcortical structures which in the normal animal easily induce spindle waves or recruiting responses (intralaminar and midline nuclei of the thalamus and some association nuclei). In contrast, stimulation of other cortical and subcortical structures which are much less effective in inducing spindles and recruiting responses, are also less effective in precipitating bilaterally synchronous epileptiform discharges. Topical application of penicillin to subcortical sites, particularly to the thalamus, including the intralaminar and midline nuclei, fails to elicit any form of epileptic discharge (except for penicillin application to the amygdala).

The distribution of C_{14} penicillin in different brain structures at the time of onset of generalized epileptic discharges following intramuscular and bilateral diffuse cortical application of this drug, confirmed the likelihood of a cortical onset of the epileptic activity. With the latter type of penicillin application, there was a significantly higher uptake of this drug by cortical grey matter as compared to that achieved by subcortical grey matter structures (thalamus and caudate nucleus) or by subcortical white matter (internal capsule), ($p < 0.02$).

Following microinjection of C_{14} penicillin to the thalamus, no epileptic activity was recorded from subcortical or cortical structures provided there was evidence to suggest that this drug remained confined to the injection site and did not contaminate the cortex. The uptake of penicillin in these experiments was significantly higher in thalamic than in cortical or other subcortical structures ($p < 0.02$). These experiments again support a cortical rather than a subcortical site of the epileptic alteration of neuronal behavior in feline generalized penicillin epilepsy. They also confirm the fact that subcortical structures such as the thalamus are probably insensitive to the epileptogenic action of penicillin. However, it is likely that penicillin increases the excitability of the thalamic and extra-thalamic neuronal pool which would account for the presence of rhythmic subcortical discharges occurring synchronously with a cortical epileptic burst when penicillin is given intramuscularly. This would also explain their absence when penicillin is applied diffusely to the cortex.

From these data it can be concluded that the epileptogenic alteration of neuronal behavior in generalized penicillin epilepsy of the cat resides in the cortex, but that subcortical structures involved in spindle generation and recruiting responses are the most potent triggers for precipitating bilaterally synchronous epileptic discharges. A functional alteration of thalamic structures is clearly insufficient for precipitating bilaterally synchronous epileptic discharges.

These findings also indicate that the basic pathophysiological mechanism of feline generalized penicillin epilepsy, and by extrapolation, probably that of human generalized corticoreticular ("centrencephalic") epilepsy, is based on a mild, diffuse cortical epileptogenic state, with subcortical structures involved in spindle generation and recruiting responses, (particularly the "thalamic reticular system") acting as the most potent triggers of generalized bilaterally synchronous spike and wave discharge. Thus both cortical and subcortical mechanisms are important for the elaboration of generalized bilaterally synchronous spike and wave discharge.

CLAIM TO ORIGINAL WORK

The clinical and EEG features of generalized penicillin epilepsy in the cat resemble human myoclonic petit mal. This is an interesting model which is suitable for the study of some of the pathophysiological mechanisms proposed to explain the origin of the widespread epileptiform discharges in the human condition. The research described in this thesis was designed to clarify the role of cortical and subcortical (particularly thalamic) structures in the genesis of generalized and bilaterally synchronous spike and wave activity. The experimental results, led to the conclusion that widespread epileptic discharge in feline generalized penicillin epilepsy is dependent upon a diffuse mild epileptogenic state of the cortex and not of the thalamus or of the subcortical structures. It was however, apparent from our experiments that the most potent trigger mechanism for precipitating bilaterally synchronous spike and wave discharge resided in those subcortical structures which in the normal animal are involved in spindle genesis and recruiting responses. These structures are mainly centered upon the intralaminar and midline nuclei of the thalamus and constitute the so-called "thalamic reticular system".

By using radioactive C_{14} penicillin it was possible to demonstrate the distribution of the drug within the brain resulting from the three modes of penicillin application used in this study and to correlate these findings with the electrophysiological data. These correlations fully supported the interpretation given to the electrophysiological data.

By ascribing significant and distinctive roles to cortical and subcortical structures in the genesis of generalized and bilaterally synchronous spike and wave activity, it may be hoped that the findings reported in this thesis may prove to be applicable to the human condition it mimicks and thus may help to lay to rest the old controversy between the "centrencephalic" as opposed to the "cortical" hypothesis of generalized seizures.

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