MECHANISM OF SUSCEPTIBILITY OF RATS TO AUDIOGENIC SEIZURE

by

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I hereby recommend that this dissertation prepared under my direction by Bruce Richard Duplisse

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ABSTRACT

The seizure response of audiogenic seizure susceptible (AGS) animals is influenced by the nature of the sound to which they are exposed. Several modes of sound generation were evaluated and electric doorbells were found to be the most effective for producing seizures in AGS rats. The effect of repeated sound stimulation on responsiveness to audiogenic seizures was also studied. It was found that a single sound stimulation per week for 5 weeks produced no change in seizure response. A single stimulation per day produced progressively weaker responses over several weeks and 10 or 20 closely repeated sound stimulations per day caused a high percentage of AGS rats to become refractory to audiogenic seizure within several days. In research involving AGS animals the optimal sound source should be used and testing should be appropriately spaced to avoid unintentional development of refractoriness.

The mechanism of susceptibility to sound-induced seizure has not been elucidated. It is suggested that AGS rats have one or more defects in the auditory pathway which allow auditory impulses to spread beyond auditory neurones to cause running and convulsions. Bilateral lesions of the ventral cochlear nucleus (VCN) or the inferior colliculus (IC) blocks audiogenic seizures, but ablation of the superior olivary complex (SOC) or the medial geniculate (MG) does not. Thus a defect in the IC may be responsible for susceptibility to audiogenic seizure. Additional studies were conducted to support this contention. For example, electrical

stimulation of the IC of AGS or non-AGS rats produces a seizure pattern identical to audiogenic seizure, whereas stimulation of the MG or reticular formation (RF) produces different patterns. Further, the electroseizure threshold in the IC of AGS rats is 7-fold lower than that in non-AGS rats. This lower threshold is not a reflection of a generally more sensitive central nervous system in AGS rats, since the electroseizure thresholds of the MG and RF in AGS rats are no different than those in non-AGS rats.

AGS rats rendered refractory to audiogenic seizures by repeated sound stimulation regained sensitivity to audiogenic seizures following treatment with isoniazid, a drug that depletes γ-aminobutyric acid (GABA). RO 4-1284, a drug which depletes catecholamines and 5-hydroxytryptamine, failed to reverse the refractoriness. These observations suggest that deficit of GABA in the IC may be the defect responsible for susceptibility to audiogenic seizures.

Bilateral injection of bicuculline (BCC), a GABA antagonist, into the IC of AGS and non-AGS rats produces a seizure pattern identical to an audiogenic seizure. Because AGS rats are more sensitive to BCC it is suggested that they have a deficit of GABA in the IC and therefore have less inhibitory activity in the IC than non-AGS rats. Furthermore, bilateral injections of GABA into the IC produce blockade of seizures induced by sound or injection of BCC into the IC. The anti-audiogenic seizure effect of GABA in the IC may represent temporary repair of a deficiency in the IC of AGS rats and may reflect the modulatory role of GABA in this and possibly other auditory nuclei. The results of this investigation provide

evidence that a deficit in the GABA inhibitory system in the IC is an important factor responsible for susceptibility to audiogenic seizure.

CHAPTER I

GENERAL INTRODUCTION

Seizures in Man

Occurrence

Approximately 0.5 to 1.5% of the people living in the United States are afflicted with epilepsy (Geist, 1962, p. 15; DiPalma, 1965; Adams, 1970; Scott, 1973). A rigorous investigation conducted by Stanhope, Brody, and Brink (1972) on the island of Guam revealed an incidence of epilepsy among the population of Guam similar to that in the United States. One investigator has estimated recently that, throughout the world, there are 32 million epileptic people (Scott, 1973, p. 5).

Etiology

Although Hippocrates was the first to report that epileptic seizures originated in the brain, it was not until the seventeenth and eighteenth centuries that this central theory was actually accepted (Penfield and Jasper, 1954, pp. 3-19; Geist, 1962, p. 13; Scott, 1973, pp. 2-4). Epileptic seizures may have existed since the beginning of man, and perhaps even earlier, since the beginning of certain animals (Penfield and Jasper, 1954, p. 3; Geist, 1962, p. 13). Epilepsy, a Greek word, means "to fall upon or seize from without." This meaning reflects the view of early man that an epileptic fit was the result of spiritual or demonological intervention (Penfield and Jasper, 1954,

pp. 20-21; Scott, 1973, pp. 3-8). Epilepsy displays a wide variety of signs and symptoms but a common feature in all cases is the occurrence of excessive neuronal discharge in the central nervous system (CNS). Clinically, epilepsy is classified into "symptomatic" epilepsy which can be traced to a known causative factor and "essential" or "idiopathic" epilepsy which originates from an obscure or unknown etiology. Woodbury and Fingl (1975) define epilepsy today as a group of recurring disorders of the central nervous system (CNS) which produce spontaneous seizures associated with loss or disturbance of consciousness, usually convulsions, and sometimes autonomic hyperactivity, and are always associated with abnormal and excessive EEG discharges. Millichap (1972) suggests that a more rational classification of epilepsy, based upon drug therapy, consists of four groups: major, minor, psychomotor, and focal. Some of the more common types of epilepsy are briefly reviewed below.

Major Seizure Patterns. Grand mal epilepsy is the most common form of epilepsy and accounts for 50% of epileptic convulsions (DiPalma, 1965; Scott, 1973, p. 23). Grand mal seizure originates symmetrically in the brain and this epileptic discharge spreads from the original foci to more distant sites and often encompasses most of the brain (Penfield and Jasper, 1954, p. 29; Gibbs and Stamps, 1958, pp. 28-31). This epileptic attack is frequently preceded by a sensory or motor phenomenon known as an "aura." Onset of the seizure is characterized by sudden loss of consciousness, tonic convulsions followed by clonic activity, and deep sleep. When consciousness is restored, there is no recollection of the episode (Gibbs and Stamps, 1958, pp. 28-31; Adams, 1970; Scott, 1973, pp. 23-25).

Minor Seizure Patterns. Petit mal epilepsy is characterized by a sudden, brief lapse of consciousness which occurs without warning and lasts only a matter of seconds. Convulsive motor activity and postictal signs and symptoms are absent or slight (Gibbs and Stamps, 1958, pp. 23-25; Adams, 1970; Scott, 1973, pp. 25-26).

Psychomotor Seizure Patterns (Temporal-Lobe Seizures). Psychomotor epilepsy is the second most common form of epilepsy and is identifiable by the presence of one or more of three distinct signs or symptoms known as the "psychomotor triad." The first sign is an "aura" which is a complex hallucination such as a vivid recollection of a past experience. The second sign is inappropriate stereotyped behavior, such as walking, rubbing, or disrobing. The third sign of the "psychomotor triad" is convulsive movements which affect the motor activity of the jaw, tongue, head and eyes but rarely the limbs. Postictal signs and symptoms are characterized by sleepiness and/or headaches and little or nothing is remembered of the seizure (Gibbs and Stamps, 1958, pp. 39-42; Adams, 1970; Scott, 1973, pp. 26-28).

Focal Seizure Patterns. Jacksonian seizures are associated with a focus in the rolandic cortex and begin as clonic movements on one side of the body. The clonus begins either in the face, hand, or foot and spreads systematically over the whole side of the body. The uniqueness of this rare form of epilepsy is the maintenance of complete consciousness (Penfield and Jasper, 1954, pp. 24-25; Adams, 1970; Scott, 1973, p. 26).

These major clinical forms of epilepsy can be further subdivided through careful analysis of the symptoms and the electroencephalogram.

The existence of one of these clinical forms of epilepsy does not preclude the presence of another form in the same patient. Frequently, the less severe forms of epilepsy are mixed with bouts of grand mal epilepsy (Gibbs and Stamps, 1958, pp. 28-31; Geist, 1962, pp. 16-18).

It is apparent that epilepsy is a complex disorder with a wide spectrum of possible symptomatology. At the present time drug therapy is merely symptomatic. Epileptic research seeks to develop better drugs to improve symptomatic management of this neurological disorder, and to reveal mechanisms involved in seizure activity through which a cure may be realized (Killam, 1969).

Animal Models of Convulsive Disorders

Research on humans is subject to many restrictions, therefore, animal models must be employed that will accurately depict the various clinical forms of epilepsy in man. These models utilize electrical, chemical, or surgical techniques to induce seizures. Other models rely upon the existence of strains of animals in which environmental factors, such as light and sound, may trigger a seizure (Toman, 1964; Killam, 1969).

In view of the paucity of information available on mechanisms of seizure susceptibility, data from animal studies which involve experimentally-induced seizures cannot be readily extrapolated to human disease (Killam, 1969). Animal models have been equated to specific forms of human epilepsy purely on an empirical basis. The pattern of protection afforded by a carefully selected group of clinically

distinguished epileptic drugs establishes the criterion for the differentiation of the animal model (Toman, 1964).

Each animal seizure model provides tests by which the effectiveness and specificity of new drugs for the management of epilepsy may be judged. In addition, each of these models provides techniques whereby the mechanisms of seizure initiation and propagation may be studied.

Seizure Models Based on Electrical Stimulation

Electroconvulsive shock, as a screening technique for antiepileptic drugs, was developed in 1937 by Putnam and Merritt and by Spiegil. This technique has been involved in the discovery of most modern antiepileptic drugs (Stille and Sayers, 1967).

Maximal electroshock seizures have proven useful in screening for drugs effective against grand mal epilepsy. In maximal electroshock seizures, a supramaximal current which produces tonic contraction of all skeletal muscles is delivered through corneal or pinnal electrodes. Drugs effective against grand mal epilepsy produce blockade of the tonic extensor phase of the seizure. Unfortunately, many drugs which lack antiepileptic activity also block tonic extension (Swinyard, 1949; Toman, 1964; Stille and Sayers, 1967).

Minimal electroshock seizures are provoked by the same methods which are utilized for maximal electroshock seizures, but a much lower current is required to trigger a minimal response. During the minimal seizure, the animal typically displays alternating limb clonus. Protection in this test is conferred by drugs effective against petit mal epilepsy. Low frequency electroshock seizures, a modification of minimal

electroshock seizures, was initially believed to be an animal model for psychomotor epilepsy. However, drugs effective against petit mal epilepsy block seizures produced by low frequency electroshock. For this reason, low frequency electroshock is not considered a specific test for drugs effective against psychomotor epilepsy (Toman, 1964).

Seizure activity can also be provoked by electrical stimulation of discrete brain areas through implanted electrodes. While the electroshock seizure threshold is useful in drug screening, stimulation of discrete brain areas is useful in mechanistic and anatomical research. Production of convulsions by cortical stimulation was first demonstrated in 1870 by Fritsch and Hitzig. Since that time, seizure production has been associated with a variety of brain structures which include the globus pallidus, subthalamus, tegmentum, and portions of the bulbar reticular formation (Penfield and Jasper, 1954, pp. 183-227).

Electrical stimulation of the limbic structures, especially the amygdala and hippocampus, produces local after-discharges. If stimulation is repeated at intervals, the animal eventually displays behavioral motor seizures, a phenomenon termed the "kindling effect" (Goddard, McIntyre, and Leech, 1969; Racine, 1972a and 1972b; Racine, Okujave, and Chipashvili, 1972; Arnold, Racine, and Wise, 1973; Racine, Burnham, and Gartner, 1973). McIntyre and Goddard (1973) and Racine, Gartner, and Burnham (1972) suggest that the production of seizures by means of the "kindling effect" has a basic neural mechanism which may be analogous to the mechanism of learning.

Seizure Models Based on Chemical Stimulation

Many chemicals such as strychnine and pentylenetetrazol (PTZ) have been employed in seizure research. Application of these chemicals to the cortex and to subcortical sites evokes afterdischarges on the electroencephalogram.

Cortical application of strychnine produces localized excitation and spiking which generates orthodromic waves that are useful in mapping neuronal circuits. This localized seizure discharge rarely becomes generalized unless extensive areas of the brain are exposed to strychnine via the blood supply. Therefore, a generalized seizure involves more than just the initiation of a seizure focus. Strychnine has also been employed to sensitize appropriate cortical areas in animals such that sensory input will precipitate seizures (Penfield and Jasper, 1954, pp. 210-214).

Pentylenetetrazol produces considerable activation of the cortex although its primary effect is upon the diencephalon. The anticonvulsant effects of a drug is demonstrated by its ability to block seizures produced by PTZ or by its ability to elevate the intravenous dose of PTZ necessary for causing seizure or death in an animal (Penfield and Jasper, 1954, pp. 213-216; Toman, 1964). Pentylenetetrazol sensitizes animals to sensory induced seizures and serves as a diagnostic aid in suspected epilepsy (Penfield and Jasper, 1954, pp. 216-217; Scott, 1973, pp. 69-70).

Seizure Models Based on Cortical Lesions

Spontaneous seizures can be evoked in animals through cortical lesions. Mechanical trauma to the cerebral cortex of experimental

animals is the most direct method which produces cortical injury; however, this technique yields an unsatisfactory low incidence of seizures. Therefore, other techniques, such as cortical freezing and cortical drug application, which increase the rate of seizure incidence have been devised (Penfield and Jasper, 1954, p. 220; Pollen, 1973). Production of chronic epileptic foci through the application of alumina cream to the cortex was demonstrated in 1942 by Kopeloff, Barrera, and Kopeloff. Other investigators have demonstrated that production of seizure foci can result from cortical application of other compounds, such as cobalt powder (Dow, Fernandex-Guardiola, and Manni, 1962; Goldberg et al., 1972), penicillin (Walker and Johnson, 1945; Gutnick and Prince, 1971), and tungstic acid gel (Takahashi, Yamauchi, and Hirabaysaki, 1972). An interesting observation in these cortical models is that induction of a lesion results in a "mirror" focus in the same structure of the opposite hemisphere.

Seizure Models Based on Genetic Determinants

Most seizure prone animals employed in convulsive disorder research demonstrate a form of reflex epilepsy which utilizes a sensory modality as the external initiator of seizure activity. In 1965, Killam and Naquet found that a baboon, Papio papio, from Senegal experiences seizure activity in response to a flickering light (Killam, 1969; Naquet, 1973). Since this phenomenon bears a remarkable similarity to "photic" epilepsy in humans (Penfield and Jasper, 1954, pp. 491-493; Larsen, 1969), considerable characterization of this model followed in the wake of this discovery (Killam, 1969; Meldrum, Naquet, and Balzano, 1970; Stark,

Killam, and Killam, 1970; Meldrum and Naquet, 1971; Naquet, 1973). However, inconsistencies in sensitivity and severity of seizures within this model remains a major difficulty (Killam, 1969).

Sound induced seizures are most commonly encountered in rats and mice. Audiogenic seizure susceptible (AGS) animals have proven extremely useful in the investigation of mechanisms of initiation and spread of seizure discharge. Although sensory stimulation induced seizures in animals may not be identical to epilepsy in humans, it is noteworthy that epileptic fits may be triggered by common stimuli, such as light and sound or internal influences such as pain and emotion, in certain epileptic patients. Possible anatomical and/or biochemical defects in the CNS may render these epileptic individuals unable to cope with certain normally tolerable experiences.

With sound stimulation, audiogenic seizure-prone rats and mice typically respond with a preliminary running phase(s) which usually culminates in a seizure (Lindsley, Finger, and Henry, 1942). The sound source that evokes this convulsive response is frequently an electric doorbell.

In animals, seizure activity induced by common environmental factors, such as light or sound, are believed to be more intimately associated with human idiopathic epilepsy than are seizures induced by massive doses of electricity or drugs. Initially, the uniqueness of the behavioral response of the AGS animal is difficult to reconcile with all of the clinical signs and symptoms of human epilepsy. In these animals, the convulsion is invariably preceded by a running attack which is considered by some investigators to be a part of the seizure process

(Lindsley et al., 1942; Finger, 1947; Boggan, 1973; Jobe, Picchioni, and Chin, 1973). The running attack may be the result of a localized paroxysmal discharge which breaks through the initial inhibitory barriers in some animals and becomes generalized, thus yielding tonic and clonic convulsions. Audiogenic seizure susceptible animal may serve as a useful model for the study of epileptic mechanisms and for the elucidation of mechanisms involved in the control of electrical activity in the brain (Hamburgh and Vicari, 1960).

Abnormalities in inhibitory neurotransmitter systems have been investigated for possible epileptogenic involvement. Audiogenic seizure susceptible animals have been employed to help elucidate the role of certain putative neuromediators on seizure susceptibility and seizure severity. Unfortunately, the literature is replete with studies on seizure activity in which the terms seizure susceptibility and seizure severity are not defined and are frequently used interchangeably. Since seizure susceptibility and seizure severity may involve distinctly different mechanisms, interpretation of data must be based on a clear distinction between these two parameters. In this report seizure susceptibility refers to the ability of an animal to exhibit a convulsion, whereas seizure intensity refers to the relative rigorousness of the convulsion.

Putative Neuromediators in Seizure Activity

Catecholamines

Reserpine represents one of the first extensively studied drugs on seizure activity. Reserpine reduces electroshock and chemoshock seizure thresholds (Chen, Ensor, and Bohner, 1954; Lessin and Parks, 1959;

Chen, Ensor, and Bohner, 1968; Azzaro et al., 1972) and enhances the frequency and intensity of audiogenic seizures (Lehmann, 1967; Schlesinger, Boggan, and Freedman, 1968). Reserpine is also reported to afford protection against audiogenic seizures at low doses (Schlesinger et al., 1968). Reserpine reduces the levels of many putative neuromediators in the CNS such as norepinephrine (NE), dopamine (Holzbauer and Vogt, 1956; Paasonen and Vogt, 1956; Carlsson et al., 1957) and 5-hydroxytryptamine (5-HT) (Pletscher, Shore, and Brodie, 1955). Research with reserpine has implicated these putative neuromediators as regulators of audiogenic seizure intensity (Lehmann, 1967; Schlesinger et al., 1968), but not as a determinant of audiogenic seizure susceptibility. Lehmann and Busnel (1963) report that reserpine fails to transform non-AGS mice into AGS animals. A similar observation was noted in rats in this laboratory by Jobe et al. (1973).

In audiogenic seizure susceptible rats, intracerebroventricular injections of dopamine significantly reduce audiogenic seizure severity (Jobe, 1970). Elevation of brain dopamine levels by drug treatment attenuates audiogenic seizures (Boggan and Seiden, 1971); however, Jobe et al. (1973) report that NE plays a more dominant role than dopamine in attenuating audiogenic seizure. In a more recent study, Bourn (1974) found that dopamine had no functional role in the modulation of audiogenic seizures.

Intracerebroventricular (Jobe, 1970) and intracerebral (Schlesinger, Stavnes, and Boggan, 1969) injections of NE drastically reduce audiogenic seizure severity. Drug induced increases in brain levels of NE decrease the severity of audiogenic seizures (Lehmann, 1967;

Schlesinger et al., 1969; Jobe, Picchioni, and Chin, 1969; Boggan, Freedman, and Lovell, 1971; Jobe et al., 1973) while decreases in brain NE levels exacerbate the seizure response (Lehmann, 1967; Bourn, Chin, and Picchioni, 1972; Jobe et al., 1973). Lehmann (1967) emphasizes that the amount of NE at the receptor site rather than the total brain concentration represents the essential factor. This theory provides an explanation for the observation that NE concentration may be reduced substantially and have no effect on seizure severity; however, a slight additional decrease in the brain level of NE enhances seizure severity. A small pool of NE appears to be responsible for enhancement of audiogenic seizure severity and may represent the immediate determinant of the NE concentration at the receptor site (Jobe et al., 1969).

5-Hydroxytryptamine (5-HT)

Intracerebroventricular injections of 5-HT attentuate audiogenic seizures (Schlesinger et al., 1969; Jobe, 1970). Elevation of 5-HT in the brain decreases audiogenic seizure severity (Lehmann, 1967; Schlesinger et al., 1969; Jobe, 1970; Boggan and Seiden, 1973), whereas decreased 5-HT brain levels enhance severity of audiogenic seizures (Alexander, Kopeloff, and Alexander, 1971; Laird, Chin, and Picchioni, 1974). Circadian fluctuations in the intensity of audiogenic seizures vary inversely with circadian fluctuations in 5-HT brain levels (Schreiber and Schlesinger, 1971; Laird, 1974).

γ-Aminobutyric Acid (GABA)

Although norepinephrine and 5-HT appear to be inhibitory modulators of audiogenic seizure severity they do not appear to be involved

in determining seizure susceptibility (Jobe et al., 1973). Hence, other factor or factors may be responsible for determining audiogenic seizure susceptibility. γ-Aminobutyric acid (GABA), a potent inhibitory substance in the CNS (Baxter, 1970), has been reported to be synthesized at a depressed rate in the cerebral cortex of one strain of AGS mice (Al-Ani et al., 1970). Changes in brain levels of GABA have been associated with the onset of audiogenic seizure susceptibility in non-AGS mice that were exposed to sound at a critical age ("priming") in order to render them seizure susceptible to sound (Sze, 1970). Intracranial administration of GABA in AGS mice diminishes significantly seizure susceptibility (Schlesinger et al., 1969). Protection against audiogenic seizures is afforded by drugs which raise GABA levels (Schlesinger et al., 1968; Simler et al., 1973) while drug-induced decreases in brain levels of GABA increase audiogenic seizure susceptibility (Benson and Salzberg, 1966). Stransky (1969) reported that methionine sulphoximine administration confers audiogenic seizure susceptibility to a non-AGS strain of rats and that GABA brain levels were substantially reduced throughout the susceptible period. A marked loss of audiogenic seizure susceptibility was observed with the return of GABA brain levels to normal (Stransky, 1969).

Statement of Problem

Although considerable information exists concerning the role of norepinephrine and 5-hydroxytryptamine on audiogenic seizure intensity, there is a paucity of information concerning mechanisms of susceptibility to audiogenic seizures and the involvement of putative neurochemical

mediators. The objective of this study is to investigate the mechanism(s) of susceptibility to audiogenic seizures in rats. Elucidation of these seizure mechanisms would add valuable information about the role of inhibitory systems and their neuromediators in the CNS and may contribute toward better understanding of epilepsy in man.

CHAPTER II

GENERAL PROCEDURES

Audiogenic Seizure Model

Male audiogenic seizure susceptible (AGS) rats of The University of Arizona strain were used in this investigation. All of the rats in the colony had been previously screened for audiogenic seizures and were retained in the colony because they exhibited an audiogenic response score (ARS) of no less than 2, as rated by the scoring system described below, in each of three consecutive weekly exposures to sound stimulation. The rats were housed at 25 $\pm$ 2°C, and were maintained under controlled lighting which provided equal periods of light (0600-1800 hours) and darkness (1800-0600 hours). The animals were allowed free access to water and food consisting of "Canine Checkers," Ralston-Purina Company, except during the brief periods when they were removed from their cages for experimental procedures. Animals were generally housed six to a cage. Surgically treated animals were housed individually.

Animal Selection

In studies which utilized ARS evaluation, potential test subjects were screened once every seven days, for a total of 3 trials, and only those rats with an ARS of 2.0 for all three trials were used in the experiment. These animals were randomly divided into control and test groups.

In seizure susceptibility studies, the only selection requirement was the certainty of a convulsion on every trial. Rats with the most severe seizure response were randomly assigned to groups.

Rats, 180 to 250 grams, were used in studies which did not utilize stereotaxic procedures. Rats between 280 and 320 grams were employed for stereotaxic surgery.

Characteristic Response

The typical seizure pattern displayed by untreated AGS-rats in response to sound stimulation consists of two phases, an initial preconvulsive phase and a convulsive phase. The preconvulsive components of audiogenic seizures include one or more violent running episodes which are separated by a period of refractoriness to sound. This refractory period persists for approximately 15 seconds. The convulsive phase, which is characterized by generalized clonic or tonic-clonic convulsion, occurs following a running episode. A rage reaction, characterized by running, jumping, biting and vocalizing, occasionally follows convulsions with a low ARS but is rarely associated with convulsions of high intensity. Catalepsy is especially common after the less intense convulsions but postictal depression is apparent in all animals. The depression fades gradually and normal activity resumes.

Sound Stimulation Technique

Two different sound chambers were employed for stimulation of AGS rats. The standard sound chamber (SSC) is a cylindrical chamber, 16 inches in diameter and 20 inches high, constructed of galvanized metal, and enclosed in a sound insulated wooden cabinet. A sound level

of approximately 115 db relative to 1 x 10^{-4} dyne/cm^2 was generated by two electric doorbells mounted within the metal cylinder.

Sound stimulation was initiated within 15 seconds after placement of a rat in the chamber. The sound stimulus was continued until a convulsion occurred (usually in less than one minute) or for an arbitrary maximum of 100 seconds in animals that do not convulse. Rats that do not convulse within one minute typically will not convulse despite longer periods of stimulation. All tests were conducted during the light hours (0600 to 1800 hours) and the ARS was determined for rats on an individual basis. This unit also served as a sound treatment chamber for groups of ten rats that were exposed to repeated sound stimulation in order to reduce audiogenic seizure susceptibility.

The modified sound chamber (MSC) employed a Lehigh Valley Behavioral Chamber (Model 1579-A). This unit consisted of an outer isolation chamber and an inner plexiglass chamber for animal confinement. A plexiglass plate inserted upon the grid floor minimized the danger of injury to the animal during seizures. A five inch, 8 volt, electric doorbell, which was mounted on a wooden support and positioned in the outer chamber adjacent to the animal enclosure, generated a sound intensity of approximately 90 db within the animal enclosure.

Sound stimulation was initiated 15 minutes after placement of the animal in the MSC, except when timed drug treatments compelled earlier stimulation. Sound stimulation was continued until convulsion occurred or for a maximum of 100 seconds in animals that did not convulse. Audiogenic seizure susceptible rats exposed to sonic stimulation in the SSC and MSC displayed comparable audiogenic response scores.

Evaluation of Response

The method originally described by Jobe et al. (1973) was used to quantify the intensity of seizure response. With this method the response is recorded numerically and is based on the number of running phases and the intensity of the convulsion (see Figure 1). The audiogenic response score (ARS) is directly related to the severity of convulsion as manifested by the appearance of varying degrees of tonic activity and is inversely related to the number of running episodes. In audiogenic seizure susceptibility studies an all-or-none endpoint was used. The number of animals that displayed seizures within a group, regardless of intensity, was recorded as a percentage of the total number of animals in the group.

Electrically-Induced Seizures

Maximal Electroshock Seizure Threshold

Maximal electroshock seizure threshold (MEST) was determined by the method described by Swinyard (1949). An electroshock apparatus (Hans Technical Associates, Palo Alto, Cal.), built according to the specifications of Woodbury and Davenport (1952), was used to deliver a 60 Hz alternating current of 0.2 seconds duration to the animal by means of corneal electrodes. Since a small percentage of rats do not exhibit the maximal seizure pattern, subjects were selected for this study on the basis of their ability to display a maximal seizure when subjected to 120 milliamperes of current. In determining the MEST the staircase method of Finney (1952) was used to select the current delivered to each animal. This method provides for an increment decrease in current for

ARS SCORE — RESPONSE TO SOUND STIMULATION

0 ---------- No response.

1 ---------- Running only; no convulsion.

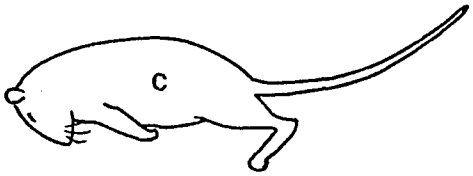
2 ---------- Two running phases separated by a refractory phase; convulsive endpoint consists of tonus of forelimbs and head followed by clonus of pinnae, vibrissae, and tail. (A)

3 ---------- Same as 2 except only one running phase and no refractory phase.

4 ---------- Two running phases separated by a refractory phase; convulsive endpoint consists of tonic flexion of neck, trunk, and forelimbs with clonus of hindlimbs. (B)

5 ---------- Same as 4 except only one running phase and no refractory phase.

6 ---------- Two running phases separated by a refractory phase; convulsive endpoint similar to 4 except hindlimbs are in partial tonic extension (i.e., tonic extension of thighs and legs with clonus of feet). (C)



7 ---------- Same as 6 except only one running phase and no refractory phase.

8 ---------- Two running phases separated by a refractory phase; convulsive endpoint similar to 4 except hindlimbs are in complete tonic extension (i.e., animal in maximal convulsion). (D)

9 ---------- Same as 8 except only one running phase and no refractory phase.

Figure 1. Description of Audiogenic Response Scoring System.

every positive response (maximal seizure) and an increment increase in current for every negative response. The endpoint for maximal seizure consisted of complete extension of the hindlimbs. This seizure endpoint was similar to audiogenic convulsion type D depicted in Figure 1.

Minimal Electroshock Seizure Threshold

Minimal electroshock threshold (EST) determinations employed the electroshock apparatus described for MEST with the exception that ear clip electrodes were used instead of corneal electrodes. Current intensity was reduced but current duration of stimulation was maintained at 0.2 seconds. The staircase method of Finney (1952), as described for MEST, was also employed in this phase of the investigation. Minimal electroshock seizure was characterized by evidence of part or all of the following manifestations: facial, lower jaw and/or forelimb clonus and rhythmic movements of ears and vibrissae. These convulsive effects usually occurred without loss of posture.

Localized Bipolar Stimulation of the Brain

Bipolar electrical stimulation was conducted in a Lehigh Valley Isolation Chamber which contained a cylinder (20 inches in diameter by 3 feet high) that was fabricated from a two inch thick section of foam rubber. A low noise, multiconductor wire (Gavitt Wire and Cable Company, No. 26, 7/34) that was coiled about a low tension spring extended from the top of the chamber to an amphenol connector on the head of the rat. A second amphenol fitting connected the upper end of this wire to a mercury coupler (Laboratory Concepts Inc., Bronx, New York) which was centered directly above the chamber. The mercury coupler was connected to a

multiple channel junction box (Grass Instrument) which was in turn connected to wide band preamplifiers on a Grass Polygraph Model 7B. This arrangement decreased electrical noise which originated from cable movement and allowed the animal complete freedom of movement.

A specific brain structure was electrically stimulated by application of a voltage across both sides of a bipolar electrode that had been stereotaxically implanted in the structure. Electric current of a specific intensity was generated by means of a Grass Stimulator, Model S4, and was delivered to the rat via the rotary mercury coupler. Recording electrodes from various brain regions were connected to a Grass Polygraph, also through the rotary mercury coupler. Stimulation parameters were as follows: biphasic pulses of 2.0 milliseconds duration, delay of 0.5 milliseconds and a frequency of 60 Hz. Application of a potential difference at the tip of a bipolar electrode embedded in the brain established a current flow in a discrete area of the brain. Stimulation was continued until a tonic seizure occurred or for a maximum of 100 seconds. One volt represented the initial intensity applied. If stimulation failed to evoke a tonic seizure, the stimulation was repeated stepwise at a progressively higher voltage until a seizure occurred. Since 20 volts was selected as the upper limit, seizure threshold was indicated as greater than 20 volts if this voltage intensity failed to induce tonic activity.

Rats were tested individually in the following manner: the rat was held firmly against the floor of the chamber while the amphenol plugs on the rat and lead wire were connected. The rat was allowed a 20 minute adaptation period before the initial stimulation. Successively more

intense stimulations were repeated at 20 minute intervals until a tonic convulsion occurs or until a maximal stimulus of 20 volts was reached. In certain animals normal behavioral activity did not return during the 20 minute interval. In such cases the rest interval was extended and stimulation was not repeated until normal behavioral activity had returned. Following testing the rat was returned to its home cage and not tested again for at least 48 hours. Data was presented as the mean seizure threshold for the group.

Chemical Injections into the Brain

Aqueous solutions of drugs were injected into specific bilateral CNS structures in a 0.5 microliter volume in each side. The injection system for each side consisted of a graduated 5 or 10 microliter Hamilton syringe that was joined to an injection needle by a segment of polyethylene tubing, size PE 20. The injection needle was inserted into a permanent intracranial cannula that was stereotaxically implanted in a specific brain structure by the method presented in Appendix B. Following insertion of the injection needles, the animal was left undisturbed for 15 minutes at which time the requisite volumes of test solution were injected. Each pair of bilateral injections was administered within a ten second interval.

These tests were conducted in the modified sound chamber with the injection tubings entering the chamber through small matched openings through the tops of the outer and inner chambers. This arrangement permitted remote intracerebral administration of drugs in a free moving animal. In some studies animals were subjected to sound stimulation

5 minutes following injection. In other studies animals were observed for 15 minutes for signs of chemically induced convulsion. Following testing each rat was returned to its home cage and was not tested again for at least 48 hours.

Surgical Preparation of Animals

Bipolar Stimulating and Recording Electrodes

The techniques for preparation and implantation of bipolar electrodes into central auditory nuclei are described in Appendix A.

Cortical Electrodes

Implantation of cortical electrodes was executed during the surgical procedure for placement of bipolar electrodes, the details of which are described in Appendix A.

Subcortical Cannulae

The techniques for preparation and implantation of subcortical cannulae are similar to the techniques used for implantation of subcortical electrodes and are described in detail in Appendix B.

Electrolytic Lesions

Brain lesions were produced by a radio-frequency electrical current from a Grass Lesion Maker LM-4, administered through an electrode implanted stereotaxically in a brain structure. This procedure is described in Appendix C.

Histological Technique

Basic histological techniques employed in this investigation were modifications of the techniques used by Humason (1967). The block of brain tissue was aligned in the plane of the reference atlas by the method of Richer (1967). Placement of lesions, cannulae, and electrodes was verified by frozen serial sections of rat brain. The atlas of Pellegrino and Cushman (1967) served as the reference. The details of this procedure are presented in Appendix D.

Statistical Procedures

The median convulsive current (CC50) for maximal and minimal electroshock seizure thresholds was determined graphically. Fiducial limits were calculated and CC50 were compared according to the method of Litchfield and Wilcoxon (1949). The Student t-test was applied to determine significant differences between means (Snedecor and Cochran, 1967). The methods of data analysis are described in Appendix E.

Preparation of Drugs

Information regarding preparation of drug solutions is presented in Appendix F.

CHAPTER III

FACTORS THAT INFLUENCE SUSCEPTIBILITY TO AUDIOGENIC SEIZURES IN RATS

Introduction

The audiogenic seizure susceptible (AGS) rat has not been thoroughly characterized. The influence of various factors such as type and intensity of sound stimuli and frequency of testing on the seizure response remains to be determined. In addition, knowledge concerning the quality of sound stimulation required for inducing seizures in AGS rats is essential for designing a sound delivery system that provides the most effective sound characteristics for seizure initiation.

Audiogenic seizure susceptibility is determined by the interplay of numerous factors. One prominent determinant is genetic predisposition which is suggested by the wide variance in susceptibility that is observed between different strains of rats and mice (Finger, 1947; Fuller and Collins, 1970). A colony of animals which possesses a high vulnerability to sound induced seizures evolves when AGS animals are mated over many generations. The genetic mechanism(s) of audiogenic seizure susceptibility are yet to be resolved. Elucidation of the genetic mechanism(s) is further complicated by the demonstration of Henry (1967) that mice of a "resistant" strain are rendered sound sensitive when these animals are exposed to sound stimulation at a "critical" age. This phenomenon, termed "priming," has been confirmed by other investigators (Fuller and

Collins, 1968; Iturrian and Fink, 1968; Alexander and Gray, 1970; Chen, 1973a).

Sound sources utilized in audiogenic seizure research have included air jet, whistle, buzzer, bell, and oscillator (Finger, 1947). A spectrum of single frequency tones was investigated by several groups and the optimum frequency for seizure production in AGS mice and for "priming" in non-AGS mice has been determined (Henry, Thompson, and Bowman, 1971; Bock and Chen, 1972). The electric doorbell is the most common sound source utilized in audiogenic seizure research (Swinyard et al., 1963; Collins, 1970; Iturrian and Johnson, 1971a and 1971b; Wada et al., 1971; Saunders et al., 1972).

The past sensory and convulsive experiences of an animal may profoundly influence its susceptibility to seizures. In mice, intense sound prior to "priming" (Chen, 1973b) or repeated sound stimulation at 6 to 12 hour intervals after "priming" prevents the development of sound susceptibility (Collins, 1970). A short period of refractoriness to sound immediately follows the occurrence of a sound induced seizure in AGS animals (Finger, 1947). Temporary refractoriness to seizure which follows a convulsive episode appears to be a general phenomenon. For example, electrical or sound induced seizures produce a temporary reduction in susceptibility to further electroshock seizures (Herberg, Tress, and Blundell, 1969). Additionally, repetitive induction of convulsions confers an extended period of refractoriness to seizure. Daily electrically induced seizures in cats elevate the electroconvulsive seizure threshold (Essig, 1968). Sound stimulation in AGS rats four times a

day for several days, causes complete loss of susceptibility to audiogenic seizures (Daliers and Rigaux-Motquin, 1968).

Chemical modification of the functional state of the nervous system may ameliorate or exacerbate seizure activity or instigate a paroxysmal discharge in normal nervous tissue. For example, convulsive doses of pentylenetetrazol or strychnine consistently produce convulsions in all animals regardless of genetic characteristics. On the other hand, subconvulsive doses of pentylenetetrazol or strychnine increase the risk of sound induced seizures in animals that are genetically susceptible to audiogenic seizures. Indeed, these drugs have been employed to sensitize animals for audiogenic seizure research (Finger, 1947). Certain compounds, which are not primarily convulsants, alter the availability of proposed neuromodulators and influence the intensity of audiogenic seizures. For example, agents such as reserpine, para-chlorophenylalanine, RO 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9, 10-dimethoxy-1,2,3, 4,6,7-hexahydro-11bH-benzo(a) quinolizine), and 6-hydroxydopamine which deplete 5-hydroxytryptamine and/or catecholamines greatly intensify audiogenic seizures.

Another proposed neuromediator in the CNS is γ-aminobutyric acid (GABA). When γ-aminobutyric acid is injected into the cerebral tissue of AGS mice, it affords protection from audiogenic seizures (Schlesinger et al., 1969). Conversely, when brain GABA levels are depressed by the administration of thiosemicarbazide AGS mice display an increases susceptibility to audiogenic seizures (Benson and Salzberg, 1966).

The objectives of this phase of the investigation are threefold: (1) to assess several sound sources and select the most effective one for

subsequent use in this investigation, (2) to evaluate different schedules of repetitive sound stimulation and determine their ability to induce refractoriness to sound-induced seizures, and (3) to treat such refractory AGS rats with several chemicals that deplete modulators of audiogenic seizure and determine whether they restore sensitivity to sound-induced convulsions.

Methods

Animals

Male AGS rats, selected according to previously described procedures (Chapter II) were used in this segment of the investigation.

Quality of Sound

Airjet. A standard laboratory air nozzle which allowed variation in air flow served as the sound source. Animals were tested individually in a metal cage (24 x 40 cm) with wire mesh on one side. The cage was positioned at a distance of 10 centimeters from the air nozzle and the screened side of the cage was aligned parallel to the flow of the air jet.

Pure Tone. Single frequency waves, delivered through a high fidelity speaker, were produced by a pure tone generator (Eico Model 377) and were adjusted to the desired amplitude by a Heath Kit amplifier. The speaker was situated directly over a glass chromatography jar which housed the animal. The speaker and chromatography jar were enclosed within a wooden sound insulated cabinet which was equipped with a viewing window.

Mixed Sound Frequencies. Mixed frequency sounds originated from either a Random Noise Generator (General Radio, Type 1390-3B, Serial No. 2814) or an electric doorbell. The bell sounds were presented to the test animals in two ways: directly and as recorded sound. Three different types of tape recorders, Uher Royal deluxe, Sony Portable TC 801A, and Sony TC 770-2 were used to record and play back the bell sounds. The output from a tape recorder or the random noise generator was presented to the animal through the speaker in the chamber described in the previous paragraph. Direct exposure to bell sounds was conducted in the standard sound chamber as described in Chapter II.

Repeated Sound Stimulation and Induced Refractoriness

The standard sound chamber (Chapter II) was used in the repeated sound stimulation studies. Animals were exposed to 100-second sound exposures ranging in frequency from once-a-week to 20 consecutive exposures over several hours. In once-a-week and once-a-day trials, the animals were exposed to the sound stimulus individually and ARS were recorded. In the 20 consecutive and 10 consecutive sound exposure studies, the animals were evaluated individually each day for ARS prior to the intensive daily repetitive sound stimulation. This sound treatment was performed on aggregated rats, up to 10 per group. In the 20 consecutive exposures, rats were subjected to sound stimulation (100-second duration) 10 times at 5-minute intervals, 5 times at 10-minute intervals, 3 times at 20-minute intervals, and twice at 60-minute intervals. In the 10 consecutive exposures, rats were subjected to sound stimulation 10 times at 5-minute intervals. In addition, one group of aggregated rats was

subjected to 25 minutes of continuous sound stimulation following daily individual evaluation of ARS.

Reversal of Refractoriness to Sound Stimulus

Isoniazid and RO 4-1284 were evaluated for their ability to restore sensitivity to sound stimulation in AGS rats that have been rendered refractory by repetitive exposure to sound stimuli. Aqueous solutions of drugs were administered intraperitoneally in a volume of 2 ml/kg. Isoniazid (INH) was dissolved in deionized water and injected in doses of 50, 100, or 200 mg/kg. RO 4-1284 was dissolved in normal saline and administered in a dose of 5 mg/kg. The audiogenic response score was determined one hour after administration of INH or RO 4-1284.

Results

Quality of Sound

Airjet. Pressurized air forced through the laboratory nozzle emits sound with an intensity of 80 to 90 decibels. Sounds from the airjet elicited typical seizures in AGS rats especially if the maximal sound intensity was rapidly attained. However, the latency period (time from onset of sound to initiation of running) was 30 to 40 seconds, as contrasted to a latency of 10 to 20 seconds typically observed in the standard sound chamber. Only 45% (4/9) of the AGS rats responded to the sound of the airjet with seizure activity while all of these animals sustained seizures in the standard sound chamber.

Pure Tone. A single sound frequency (pure tone) with an intensity in excess of 125 decibels was delivered to individual AGS rats and

the seizure response rate versus the sound frequency is presented in Table 1. Pure tones between 40 and 10,000 Hz failed to elicit seizure in any animal although sound produced by an electric doorbell induced seizures in every animal. Pure tones between 10 and 20 KHz, although tested on fewer animals, were equally ineffective for seizure production.

Mixed Sound Frequencies. Sound that was generated by a random noise generator and composed of a mixture of frequencies did not evoke seizures in AGS rats (Table 1). Magnetic tape reproductions of the bell sound were recorded and replayed through a speaker by means of a Sony Portable tape recorder (TC 801A). This sound delivery system failed to elicit a seizure in five AGS rats at a sound intensity of 115 decibels. The use of an Uher tape recorder as the sound source also failed to evoke a seizure. A Sony TC 770-2 tape recorder with Scotch 207 magnetic tape yielded a seizure incidence rate of 47% (7/15) in AGS rats and an average audiogenic response score (ARS) of 2.0. However, the latency period was 2 to 3 times longer than that obtained in the standard sound chamber.

Repeated Sound Stimulation and Induced Refractoriness

Weekly sound stimulation of AGS rats did not alter the ARS although 2 of 10 animals did not convulse at week 3 and 1 of 9 rats did not convulse at week 5 (Table 2). Daily sound stimulation in a group of six AGS rats produced no significant change in the group ARS during the first six days although 1 of 6 animals did not convulse during several trials (Table 3). As the daily sound trials continued, the mean ARS and the percent of animals that convulsed tended to decline. After 30 days of

Table 1. The Effect of Different Sound Frequencies on Audiogenic Seizure Susceptible Rats.[a]

Frequency (Hz)	Number of Rats	Percent Running	Percent Convulsed
Mixed (Bell)	6	100	100
Mixed (Random noise generator)	6	0	0
10,000	6	0	0
2,000	6	0	0
1,000	6	0	0
700	6	0	0
400	6	0	0
100	6	0	0
40	6	0	0

a. Testing was performed at sound intensity of 100-120 decibels. Stimulation was terminated after 2 minutes in animals that did not convulse.

Table 2. Effect of Single Weekly Sound Stimulation[a] on Audiogenic Response Score (ARS) and Seizure Incidence.

Week Number	Group Size	Group Average ARS ± S.E.	Percent Convulsed
1	10	2.00±0.00	100.0
2	10	1.95±0.12	100.0
3	10	1.90±0.18	80.0
4	10	2.25±0.17	100.0
5	9	2.33±0.24	88.9

a. Rats were exposed to sound stimulation for 100 seconds or until onset of convulsion.

Table 3. Effect of Single Daily Sound Stimulation[a] on Audiogenic Response Score (ARS) and Seizure Incidence.

Day Number	Audiogenic Response Score (ARS) [b]	Percent Convulsed
1	1.92±0.08	100.0
2	1.75±0.17	83.3
3	1.75±0.17	83.3
4	1.75±0.17	83.3
5	1.67±0.16	83.3
6	2.00±0.00	100.0
7	1.75±0.31	66.7
8	1.08±0.27	33.3
9	2.17±0.40	83.3
10	1.67±0.21	66.7
11	2.33±0.47	83.3
12	1.17±0.31	33.3
13	1.50±0.43	50.0
14	1.00±0.25	16.7
15	1.83±0.47	66.7
16	1.33±0.33	50.0
17	1.67±0.33	83.3
18	1.33±0.42	66.7
19	1.50±0.34	33.3
20	0.66±0.33	16.7
21	1.00±0.45	50.0
22	0.67±0.33	16.7
23	1.33±0.33	50.0
24	0.50±0.34	16.7
25	0.67±0.33	16.7
26	0.83±0.65	16.7
27	1.00±0.45	30.0
28	0.33±0.21	00.0
29	0.67±0.33	16.7
30	0.33±0.18	00.0

a. Rats were exposed to sound stimulation for 100 seconds or until onset of a convulsion.

b. Mean score ± S.E. of six rats.

daily stimulation, audiogenic seizure susceptibility in this group of rats was lost.

Marked reduction of ARS and loss of audiogenic seizure susceptibility in ARS rats was rapidly effected when rats were exposed to daily multiple sound stimulation (Tables 4 and 5). Continuous sound stimulation for 25 minutes each day resulted in a decrease in ARS and audiogenic seizure susceptibility (Table 6), but the decrease appears somewhat less than those observed during multiple daily sound stimulation. It should be noted that a significant number of rats died during multiple daily sound stimulation and during prolonged 25-minute daily stimulation.

Reversal of Refractoriness to Sound Stimulation

Administration of RO 4-1284 (5mg/kg) to AGS rats produced a significant rise in seizure intensity (Table 7). However, refractoriness to audiogenic seizures induced by repeated sound stimulations was not reversed by RO 4-1284 (Table 8). On the other hand, isoniazid (INH), which also increased audiogenic seizure intensity (Table 9), reversed the refractoriness to sound-induced seizures in 7 out of 13 rats (Table 10). All seizures sustained under the influence of INH were maximal convulsions.

Discussion

Quality of Sound

Interest in spontaneous seizures in rodents was generated by the report of Maier and Glaser (1938) that rats trained on a conditioned avoidance schedule sustained spontaneous seizures when these rats were

Table 4. Effect of 20 Daily Sound Trials[a] Upon Audiogenic Response Score (ARS) and Seizure Incidence.

Day Number	Group Size	Average ARS±S.E.	Percent Convulsed
0	13	2.11±0.15	100
1	10	1.00±0.30	40
2	9	0.11±0.11	0
3	9	0.22±0.22	11
4	9	0.22±0.22	11

a. Animals were exposed to 100 seconds of sound 20 consecutive times each day at ten 5-minute intervals, five 10-minute intervals, three 20-minute intervals, two 60-minute intervals. Sound treatments were administered immediately following determination of ARS.

Table 5. Effect of 10 Daily Sound Trials[a] Upon Audiogenic Response Score (ARS) and Seizure Incidence.

Day Number	Group Size	Average ARS	Percent Convulsed
0	34	2.0	100
4	23	0.0	0

a. Animals were exposed to 100 seconds of sound 10 consecutive times each day at ten 5-minute intervals.

Table 6. Effect of Single Long Daily Stimulation[a] on Audiogenic Response Score (ARS) and Seizure Incidence.

Day Number	Group Size	Average ARS±S.E.	Percent Convulsed
0	12	2.71±0.38	100
1	7	2.46±0.20	100
2	7	1.43±0.43	29
3	7	1.14±0.51	29
4	7	0.86±0.34	29

a. Animals were exposed to 25 minutes of continuous sound stimulation once a day following determination of ARS.

Table 7. Effect of RO 4-1284 on Audiogenic Response Score (ARS) of Non-refractory AGS Rats.

Rat Number	Pretreatment ARS	RO 4-1284[a] ARS
2	3	4
3	1	8
4	1	4
5	3	8
6	3	8
7	1	8
8	2	4
Average±S.E.	2.0±0.4	6.3±0.8

a. Audiogenic Response Score one hour after intraperitoneal administration of RO 4-1284, 5mg/kg.

Table 8. Effect of RO 4-1284 on Audiogenic Response Score (ARS) of Refractory Audiogenic Seizure Susceptible Rats.[a]

Rat Number	Pretreatment ARS	RO 4-1284[b] ARS
3	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
4	0	0
5	0	0
Average	0	0

a. Audiogenic Response Score one hour after intraperitoneal administration of RO 4-1284, 5mg/kg.

b. Rats were rendered refractory to audiogenic seizures by 20 sound stimulations during the preceding day.

Table 9. Effect of Isoniazid on Audiogenic Response Score (ARS) of Non-refractory AGS Rats.

Rat Number	Isoniazid (mg/kg)	Pretreatment ARS	Isoniazid[a] ARS
6	200	2.0	9.0
7	200	2.0	9.0
1	100	2.0	9.0
2	100	2.0	9.0
3	100	2.0	9.0
4	50	2.0	1.5
5	50	1.5	2.0

a. Audiogenic Response Score one hour after intraperitoneal administration of isoniazid.

Table 10. Effect of Isoniazid on Audiogenic Response Score (ARS) of Refractory Audiogenic Seizure Susceptible Rats.[a]

Rat Number	Isoniazid (mg/kg)	Pretreatment ARS	Isoniazid[b] ARS
1	200	0	0[c]
2	200	0	9
3	200	0	0[c]
4	200	0	9
5	200	0	0
6	200	0	9
7	200	0	9
8	100	0	0
9	100	0	9
10	100	0	0
11	100	0	9
12	100	0	9
13	100	0	0

a. Rats were rendered refractory to audiogenic seizures by 20 sound stimulations during the preceding day.

b. Audiogenic Response Score one hour after intraperitoneal administration of isoniazid.

c. Clonic twitching prior to sound stimulation.

unable to extinguish an aversive stimuli by the usual measures. These investigators concluded that some form of experimental neurosis was responsible for the convulsions since some emotional conflict appeared to act as the initiator (Maier and Glaser, 1940). This interpretation overlooked the presence of tactile and auditory stimulation which was produced by the strong air current that served as the aversive stimuli in their experiment. In pursuit of a sensory related origin for these spontaneous seizures, Morgan and Morgan (1939) demonstrated the occurrence of convulsions in naive rats which were exposed only to the sound produced by the high velocity air stream.

Since tactile stimulation intensifies the audiogenic response (Finger, 1947) direct exposure of the animal to the air flow must be avoided when an air jet is employed as a sound source. In the present experiment, the sound produced by an air jet proved to be a poor initiator of audiogenic seizures in AGS rats.

Audiogenic seizure susceptible rats, under the experimental conditions employed in the present study, do not display seizures when exposed to pure tone sound stimulation. This observation is in marked contrast to the report of Alexander and Gray (1970) that pure tone sound between 5 and 35 KHz is highly effective for seizure production in AGS mice. Several factors may account for this difference between these two species. For example, deficit of inhibitory mechanisms in AGS mice may be more severe than in AGS rats and, thus, less extensive stimulation of the auditory focus may be required to trigger a seizure response. Secondly, the sound wave frequency required for seizure production in AGS rats is much different from AGS mice and the appropriate sound frequency

for seizure production in AGS rats may not have been tested in this experiment. Although the available data cannot explain the difference in response to pure tone sound by AGS mice and AGS rats, they emphasize caution in the extrapolation of data derived from AGS mice to AGS rats without experimental verification.

Since mixed frequency sound provided by a random noise generator also failed to initiate seizures and since electric doorbells emit sound with essential characteristics for inducing seizures, magnetic tape recordings of bell sounds were made with the thought that the intensity of the reproduced bell sound could be controlled with a minimal distortion of frequency pattern. If effective for inducing audiogenic seizures, the reproduced bell sound could be used in sound intensity-seizure response studies. However, it was obvious to the ear that even the best recording of the bell sound lacked the vibrancy of the original sound. The poorest quality recordings were completely ineffective and even the recording with the best fidelity attained only limited success as an initiator of audiogenic seizures. The importance of the vibrant quality of sound in audiogenic seizure production is further emphasized by the fact that even direct exposure to the bell sound loses its effectiveness when the bell is prevented from resonating. Since the electric doorbell was the only sound source which provided predictable seizure initiation, it was retained as the sound source for all subsequent studies involving sound-induced seizures.

Repeated Sound Stimulation and Induced Refractoriness

Audiogenic seizure research usually entails repeated exposure of an animal to sound stimulation. Therefore, knowledge of the effects of repeated sound trials on audiogenic seizure response is necessary to avoid inadvertent compromise of the test procedure. Weekly sound stimulation does not alter seizure response, but daily stimulation produces diminution and eventual loss of audiogenic seizure susceptibility. Susceptibility is restored to normal within seven days following cessation of sound stimulation.

As the frequency of sound stimulation was increased to 10 or 20 exposures per day, the time interval required for loss of susceptibility greatly diminishes. However, exposure to continuous sound (25 minutes) is less effective than intermittent periods of sound. Daliers and Rigoux-Motquin (1968) also found that repeated short-interval sound trials eliminated audiogenic seizure susceptibility in AGS rats. It is noteworthy that "primed" mice which oridinarily become AGS, fail to display audiogenic seizures when they are repeatedly exposed to short-interval sound stimulation after the "priming" stimulus. Frequent exposure to sound stimulation in AGS animals may elicit a transitory compensatory increase in inhibitory capability which is maintained by each sound exposure but is lost following cessation of frequent sound stimulation. This inhibitory reaction may be mediated through alterations in the metabolism, storage, or response to endogenous neuromediators and these sound-induced changes may occur within the impaired neural system responsible for the spontaneous occurrence of audiogenic

seizures. Since frequent exposures to sound inhibit audiogenic seizures, AGS animals should not be tested more than once a week during long term studies. However, during short term studies not exceeding one week, AGS rats maintain a stable ARS when tested as often as once every other day.

Reversal of Refractoriness to Sound Stimulation

Although drugs, such as reserpine, which deplete biogenic amines enhance the intensity of audiogenic seizures, these drugs exhibit no effect upon susceptibility to audiogenic seizures (Lehmann and Busnel, 1963; Jobe et al., 1973). RO 4-1284 (5 mg/kg), a drug that produces a marked decrease in brain norepinephrine, dopamine and 5-hydroxytryptamine (Pletscher and Gey, 1963; Jobe, 1970), increases seizure severity in AGS rats (Table 7). On the other hand, RO 4-1284 does not restore sound sensitivity to AGS rats which are rendered refractory to audiogenic seizures by multiple daily sound exposure. This observation is reminescent of the inability of reserpine to render non-AGS rats sensitive to sound-induced seizures. Therefore, catecholamines and 5-hydroxytryptamine do not appear to function as determinants of audiogenic seizure susceptibility in either non-AGS or "refractory" AGS rats. Furthermore, the mechanism which prevents the development of seizures in both cases may involve the same inhibitory neuromediator.

It is suggested that γ-aminobutyric acid (GABA) functions as an inhibitory neuromediator in the CNS of vertebrates (Baxter, 1970). Depletion of brain GABA content by isoniazid (INH) is suspected to be at least partially responsible for the convulsant properties of this drug (Wood and Peesker, 1971; Wood, Peesker, and Urton, 1972; Wood and

Peesker, 1973). Wood and Peesker (1972) have formulated an equation which relates the convulsant (anti-convulsant) properties of a drug to changes induced in glutamic acid decarboxylase activity and brain GABA concentrations. In the present study, INH increases audiogenic seizure intensity and, unlike RO 4-1284, reverses the refractoriness to sound in AGS rats exposed to multiple daily sound stimulation. Inhibitory systems in which GABA serves as a neuromediator may be involved in the loss of sound susceptibility in "refractory" AGS rats. Hence, it is plausible that GABA may function as a genetically determined factor concerned with audiogenic seizure susceptibility.

CHAPTER IV

IDENTIFICATION OF NUCLEUS IN THE CENTRAL AUDITORY PATHWAY RESPONSIBLE FOR THE GENESIS OF AUDIOGENIC SEIZURE

Introduction

Since sound causes convulsions in susceptible animals primarily through the auditory system, rather than by stimulation of other sensory modalities such as vibratory or tactile senses, various aspects of the auditory system were investigated to evaluate their roles in the genesis of sound induced seizure. Of all the senses, hearing is the most sophisticated and most delicate. Although the inner ear is very small, it enables the organism to determine precisely the frequency, intensity, and location of sound. Sound is converted in the ear into nerve impulses which are transmitted through the first-order neurons of the auditory nerve to the cochlear nuclei of the medula oblongata where the first synaptic contact is made (Noback, 1967).

The neuronal cell bodies of the auditory nerve form a ganglion which is embedded in the cochlea and the axons project to the dorsal and ventral cochlear nuclei. As illustrated in Figure 2, the major auditory nuclei, in ascending order, are the ventral cochlear nucleus (VCN), the superior olivary complex (SOC), the lateral lemniscus nucleus, the inferior colliculus (IC), and the medial geniculate body (MG). Fibers from the MG project directly to the auditory cortex. All nuclei in the central auditory pathway are organized tonotopically by pitch and several

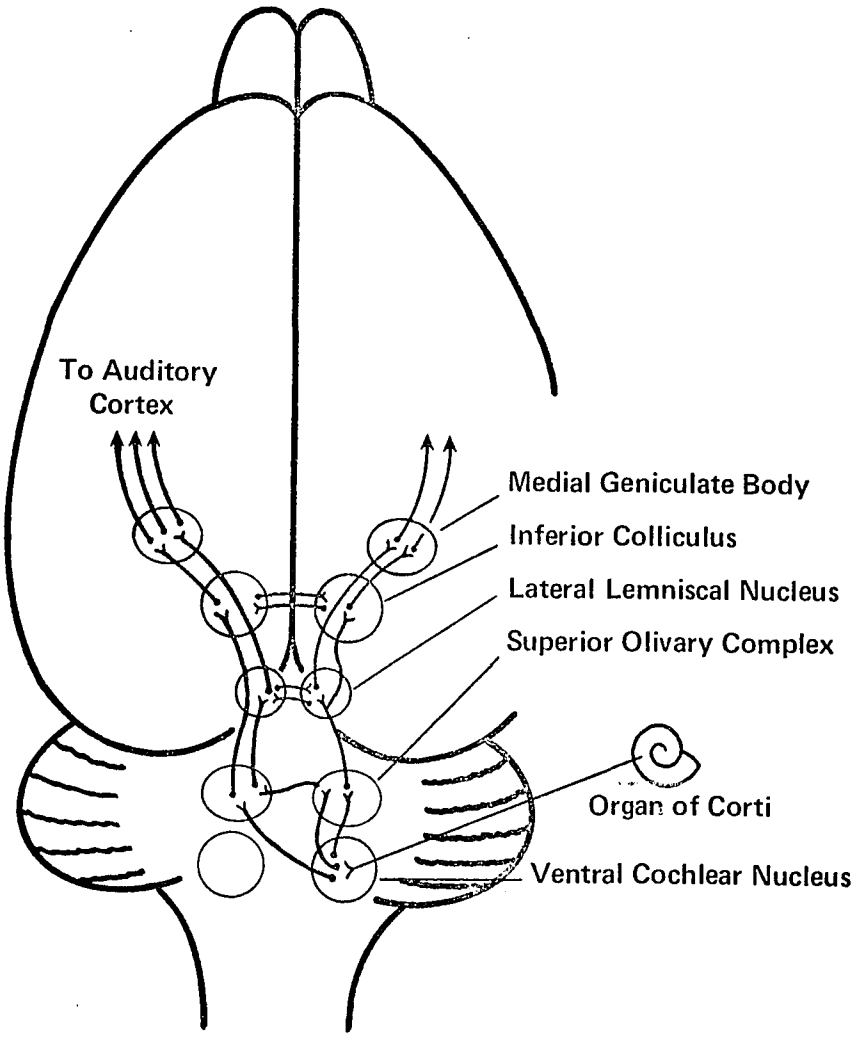


Figure 2. Diagram of the Auditory Pathway in the Rat.

Darker axons on the left side are to indicate that auditory information travels mostly, but not exclusively, to the contralateral auditory cortex.

relay nuclei serve as integration centers of sound information. Decussation of the second order neurons from the cochlear nuclei form the trapezoid body. Considerable bilateralization occurs at the level of the IC and MG via interneurons (Harrison and Warr, 1962; Noback, 1967; Osen, 1972; Tamar, 1972, pp. 165-171; Kiang et al., 1973).

The auditory system possesses considerable divergence and it is estimated in man, for example, that for every nerve fiber in the auditory nerve there are three in the cochlear nuclei and thirteen in the IC (Noback, 1967).

Descending feedback systems exist throughout the auditory pathway. The olivocochlear bundle which terminates on the hair cells of the Organ of Corti and which appears to have an inhibitory function is the most thoroughly studied auditory feedback system (Nelson and Erulkar, 1963; Noback, 1967; Gerstein, Butler, and Erulkar, 1968; Tamar, 1972, pp. 171-188; Pfalz, 1973).

Although most conscious hearing involves the cerebral cortex, many reflex auditory responses are initiated and controlled by lower centers and the IC and reticular formation (RF) may play a significant role in these reflex responses (Noback, 1967). The superior olivary complex and the IC dominate sound localization and the latter auditory nucleus is the primary center for spatial analysis of sound (Moushegian et al., 1971; Tamar, 1972, pp. 178-180). Inhibitory systems which impinge upon the lower centers of the central auditory pathway may dampen or complete eliminate certain sound impulses before they reach consciousness (Noback, 1967).

If a central auditory nucleus plays a dominant role in the genesis of audiogenic seizures, the destruction of this center should eliminate sound-induced seizures while electrical stimulation of the nucleus should emulate sound-induced seizures. Electroencephalography could assist in localization of the seizure focus. Temporary suppression of cortical function by KCl-induced spreading depression (Kesner, O'Kelly, and Thomas, 1965; Ward and Sinnett, 1971) and partial or total ablation of the cortex (Beach and Weaver, 1943; Chocholova, 1962) failed to abolish audiogenic seizures. These data suggest a subcortical mechanism for initiation of audiogenic seizures. Lesions in the amygdala and hippocampus in rats afforded only slight modification of audiogenic seizures (Kesner, 1966). Audiogenic seizures are not eradicated by bilateral lesions of the MG nor by small bilateral lesions in the IC (Koenig, 1957; Servit, 1960). On the other hand, it has been shown that total destruction of the IC eliminates audiogenic seizures in AGS rats (Kesner, 1966). Wada et al. (1970) have also substantiated that collicular lesions block audiogenic seizures in both AGS rats and in rats rendered sensitive to sound-induced seizures by thiosemicarbazide, methionine sulfoximine or pentylenetetrazol. Audiogenic seizures are abolished, likewise, by inferior collicular lesions in "primed" mice (Ward, 1971). Evoked responses in the IC are much greater in amplitude in "primed" mice than in non-"primed" mice (Saunders et al., 1972), an observation that suggests a greater reactivity in the IC of AGS animals.

Electrical stimulation of certain brain structures are known to provoke seizures. Low intensity electrical stimulation of the amygdala and hippocampus, when repeated daily, reportedly initiate seizures

(Goddard, 1967; Goddard et al., 1969). The term "kindling effect" has been applied to changes that occur during repeated electrical stimulation which increases susceptibility to seizures (Goddard et al., 1969; McIntyre and Goddard, 1973). Racine (1972b) suggests that the number of electrically induced afterdischarges is the critical factor in the "kindling effect."

Gerken (1970) reports that electrical stimulation of the auditory nuclei failed to produce convulsions; however, body orientation, aversive body movements, and running outbursts, classified collectively as "negative affective response," were seen during electrical stimulation of the central nucleus of the IC in the rat.

The objective of this phase of the investigation is to identify an area in the central auditory pathway which might account for genesis of sound-induced seizures. Identification and substantiation of such an area will require a multifaceted approach. This approach will include: (1) Determination of the effect of lesions in the auditory pathway on susceptibility to audiogenic seizure in AGS rats, (2) evaluation of the relationship between electroshock seizure thresholds and audiogenic seizure susceptibility, (3) comparison of seizure patterns produced by electrical stimulation of various central auditory structures with that induced by sound stimulation, (4) determination of electrostimulation threshold in the various auditory structures of AGS and non-AGS rats, and (5) electroencephalographic examination of seizures induced by various means.

Methods

Lesions of Central Auditory Nuclei

Various auditory nuclei of AGS rats were ablated (Grass Radio Frequency lesion maker) according to the technique described in Appendix C. The parameters for ablation of these nuclei were as follows: inferior colliculus (IC), 85 volts for 20 seconds; medial geniculate (MG), 85 volts for 25 seconds; superior olivary complex (SOC), 50 volts for 10 seconds. All lesions were verified histologically for placement and extent of destruction. The lesioned rats were tested for seizure susceptibility in the standard sound chamber 24 hours after surgery and the test procedure was repeated at daily intervals for 2 to 3 weeks.

Maximal Electroshock Seizure Threshold

Audiogenic seizure susceptible rats with an audiogenic response score (ARS) of 2.0, as determined in the standard sound chamber, were administered 100 milliamperes of current through corneal electrodes. Rats which displayed maximal seizures were randomly divided into two groups for use in the determination of maximal electroshock seizure threshold. Male Holtzman Sprague Dawley rats (ARS = 0), which displayed maximal convulsions when subjected to 100 milliamperes of current, served as the non-AGS test group.

The maximal electroshock seizure threshold was ascertained for each group of rats and was expressed as the maximal convulsive current 50 (MAX-CC50). The non-audiogenic seizure susceptible group (ARS = 0) and one AGS group (ARS = 2) were subjected to sound stimulation ten times a day (100-second sound exposure per 5 minute cycle) for four days (a

procedure for inducing refractoriness to audiogenic seizure as described in Chapter III). The other group of AGS rats which served as a control group was exposed to identical handling procedures with the exception that the sound of the bell was omitted. On the fifth day, the MAX-CC50 was redetermined for all three groups of rats.

Minimal Electroshock Seizure Threshold

The minimal seizure threshold was determined in the same manner as presented above for MAX-CC50. A lower current intensity was required for the determination of the minimal seizure threshold and the threshold was reported as the minimal convulsive current 50 (MIN-CC50) for each of the three groups before and after the sound stimulation procedure for inducing refractoriness.

Electrical Stimulation of Subcortical Brain Structures

The reticular formation (RF), MG and IC were stimulated unilaterally through bipolar electrodes and the minimal voltage required to produce convulsions was established. The seizure thresholds for the RF, MG, and IC were determined in each AGS and non-AGS rat involved in the study. A 48-hour interval was allowed between seizure threshold determinations.

Electroencephalographic Studies

Electroencephalograms were obtained from free-moving AGS rats and from AGS rats which were immobilized with IV infusion of d-tubocurarine or by varying degrees of physical restraint. Complete physical restraint was produced by tightly wrapping the entire rat with a 4-inch elastic

bandage. A partially restrained rat was confined within a plastic rat holder with its head immobilized by a clamp attached to the amphenol plug on the animal's head. Electroencephalograms were recorded before, during, and after sound stimulation and bipolar electrical stimulation of central structures in untreated animals and in those treated with RO 4-1284.

Results

Lesion Studies

Bilateral lesions in the medial geniculate (MG) were ineffective in blocking audiogenic seizures, whereas bilateral destruction of the inferior colliculus (IC) produced complete and irreversible loss of audiogenic seizure susceptibility (Table 11). Only two animals survived destruction of the superior olivary complex (SOC) due to the trauma of this technique. The rats that survived this ablation procedure responded to sound stimulation with severe seizures despite their extremely weakened condition. Bilateral lesions in the ventral cochlear nucleus (VCN) blocked sound-induced seizures, however, susceptibility returned within 7 to 10 days.

Maximal and Minimal Electroshock Seizure Thresholds

Maximal and minimal electroshock seizure thresholds for AGS rats are lower than corresponding thresholds for non-AGS rats (Table 12). AGS rats show no change in maximal or minimal electroshock seizure threshold after they were made refractory to sound-induced seizures. On the other hand, non-AGS rats showed a significant elevation in maximal electroshock

Table 11. Effect of Bilateral Ablation of Central Auditory Nuclei of Audiogenic Seizures in Rats.

Structure of Central Auditory Pathway	Number of Blocked Seizures / Number of Rats Tested	Percent Seizures Blocked
Medial Geniculate	1/7	14
Inferior Colliculi	6/6	100
Superior Olivary Complex	0/2	0
Ventral Cochlear Nucleus	4/5[a]	80

a. Seizure response returned within 7 to 10 days following surgery.

Table 12. Electroshock Seizure Thresholds for Audiogenic and Non-audiogenic Rats Before and After Repeated Sound Stimulation.[a]

Type of Rat	Median Seizure Thresholds Milliamperes (95% Fiducial Limits)	
	Maximal	Minimal
Audiogenic Rats		
Before Repeated Sound Stimulation	33.2[b] (27.0-40.8)	16.2[b] (14.7-17.8)
After Repeated Sound Stimulation	35.2[b] (32.0-38.7)	18.6[b] (14.8-23.4)
Audiogenic Rats (Control)		
Before Repeated Sound Stimulation	37.4[b] (19.9-70.3)	17.2[b] (16.1-18.4)
After Repeated Sham Sound Stimulation[c]	38.5[b] (26.2-56.3)	16.7[b] (15.4-18.1)
Non-audiogenic Rats[d]		
Before Repeated Sound Stimulation	57.0 (46.0-70.0)	25.9 (24.0-27.9)
After Repeated Sound Stimulation	83.5[b] (75.8-92.0)	28.4 (26.4-30.6)

a. Repeated sound stimulation consisted of 10 consecutive 100-second exposures to bell sound at 5-minute intervals each day for 4 days.

b. Value is significantly different from corresponding threshold value of non-AGS rats before repeated sound stimulation ($p < 0.05$).

c. Repeated sham sound stimulation is identical to repeated sound stimulation procedures such as placing animals into sound chamber for prescribed time, etc., except that actual bell sounds were omitted.

d. Holtzman Sprague Dawley Strain.

seizure threshold following 4 days of sound treatment identical to that which rendered AGS animals refractory to audiogenic seizures; however, the minimal seizure threshold was unaffected.

Electrical Stimulation of Subcortical Brain Structures

Inferior Colliculus. Unilateral electrical stimulation of the IC in rats, via bipolar electrodes, produced running activity which terminated in convulsive seizure in AGS and non-AGS rats. The seizure in AGS rats is a tonic convulsion (Type A depicted in Figure 1), whereas that in non-AGS rats is very weak and poorly defined. In AGS rats electroconvulsive threshold involving stimulation of the IC is sevenfold lower than in non-AGS rats (Table 13). Electrical stimulation of the IC at voltages below the running threshold produced either no response or occasional startle behavior. Running activity elicited by bipolar electrical stimulation of the IC must be continuous or the animals stop running. Convulsions produced by IC stimulation were typically followed by postictal catalepsy.

Reticular Formation. Stimulation of the RF, directly beneath the IC, elicited escape activity. With increasing voltage, running activity was hampered as a result of a progressive paralysis of the limbs contralateral to the stimulated side of the brain; ultimately, the animal exhibited sideward rolling movements. Severe depression which persisted for 10 to 15 minutes was prominent following stimulation, although the animal responded readily to tactile stimulation and showed no evidence of catalepsy. Audiogenic and non-audiogenic seizure susceptible rats responded in a similar fashion and at a comparable stimulus intensity.

Table 13. Electroseizure Threshold Determined by Unilateral Stimulation of Discrete Brain Structures in Audiogenic and Non-audiogenic Seizure Susceptible Rats.[a]

Type of Rats	Mean Seizure Threshold Volts±S.E.		
	Inferior Colliculus	Reticular Formation	Medial Geniculate
Audiogenic	2.1±0.6[b] (8)[c]	4.0±0.9 (5)	13.0±1.1 (5)
Non-audiogenic	15.5±0.8 (5)	4.0±1.1 (3)	11.4±1.3 (4)

a. Stimulation was performed with a Grass S-4 stimulator through implanted bipolar electrodes.

b. Significantly lower than threshold for non-audiogenic seizure susceptible rats ($P < 0.001$).

c. Number of rats tested.

Medial Geniculate. Running was seldom observed during stimulation of the MG. At low intensities, the animal raised and turned its head contralaterally. With increasing voltage, the contralateral side of the rat became increasingly involved in convulsive activity which progressed from facial twitches to clonus of the forelimb and, eventually, clonic activity in both forelimbs. The severity of the clonic activity resulted in loss of balance and the animal fell backward and symptoms resembling amphetamine stereotypy ensued upon cessation of stimulation.

Electroencephalographic Studies

Complete restraint of the animal, whether by chemical or physical means, eliminated most artifactual disturbances on the EEG. Unfortunately, complete restraint also eliminated all behavioral and electrical signs of audiogenic seizures. The electroencephalogram of a partially restrained AGS rat during sound stimulation is shown in Figure 3. As compared to the control, sound stimulation tended to increase the frequency and decrease the amplitude of cortical electrical activity and to decrease the frequency of electrical activity in the MG (control not shown). During the early stages of the tonic phase (Type A convulsion as described in Figure 1) of the convulsion, the EEG displayed an increased frequency (7 Hz to 12 Hz on tracing A) and greatly diminished amplitude especially on the cortical leads. A gradual increase in the irregularity and amplitude of the cortical EEG was observed during late stages of tonus and early stages of clonus. For a brief period following clonus, during the cataleptic stage the EEG recording from the MG exhibited large amplitude spikes which occurred at a rate of 1 per second (not shown).

Figure 3. Electroencephalogram of Partially Restrained Audiogenic Seizure Susceptible Rat During Sound-Induced Seizure.

Tracing A represents the voltage difference between the frontal cortical electrode and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing B represents the voltage difference between the parietal cortical electrode and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing C represents the voltage difference between the electrode implanted in the medial geniculate and the parietal cortical electrode at an amplification of 100 μv/cm.

Tracing D represents the voltage difference between the two tips of the bipolar electrode implanted in the medial geniculate at an amplification of 30 μv/cm.

Tracing E represents the voltage difference between the electrode implanted in the medial geniculate and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing F represents the voltage difference between the electrode implanted in the medial geniculate and the frontal cortical electrode at an amplification of 100 μv/cm.

The predominant frequency of a 2-second interval of the EEG tracing is noted directly beneath that interval in the figure.

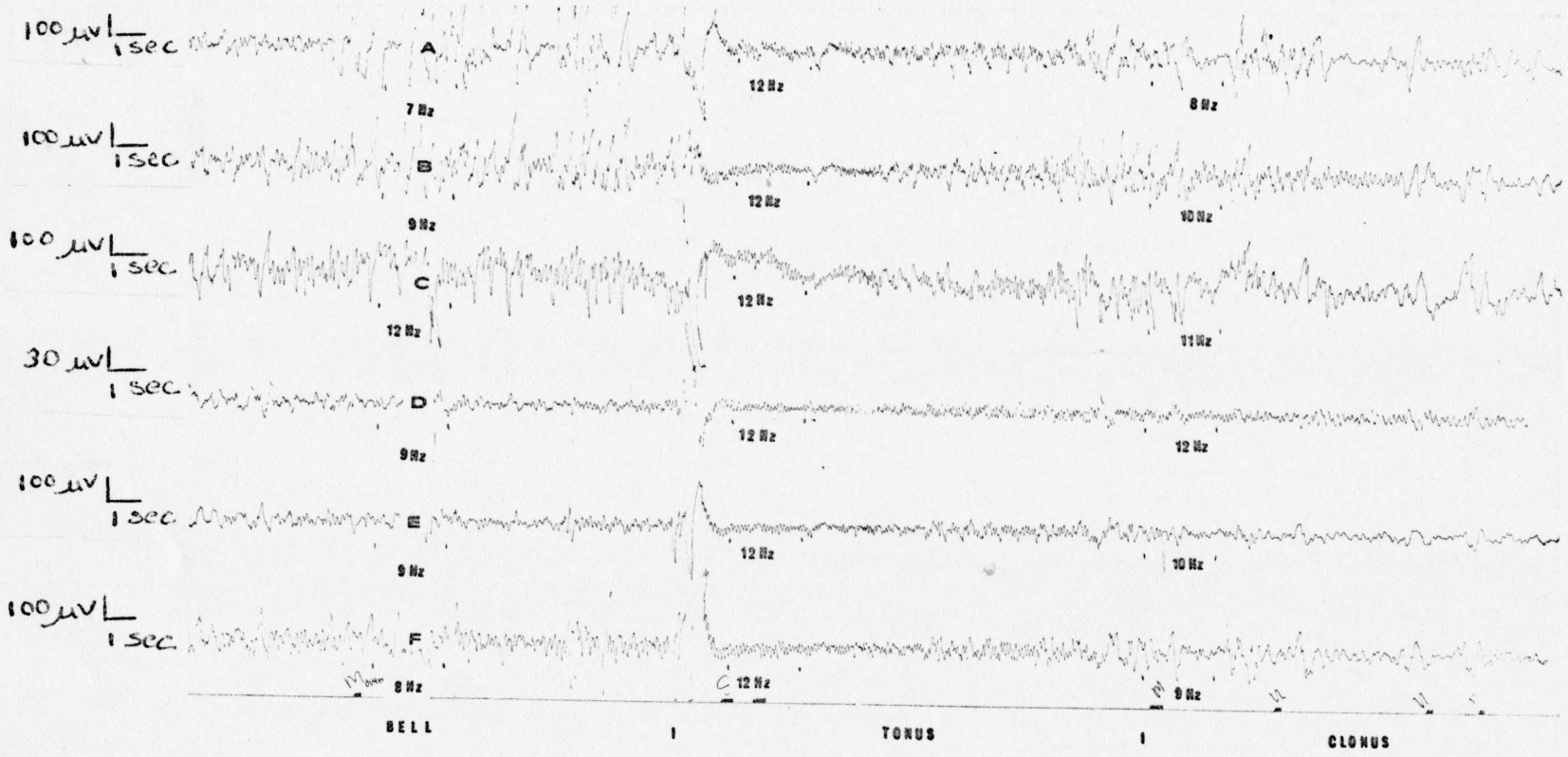


Figure 3. Electroencephalogram of Partially Restrained Audiogenic Seizure Susceptible Rat During Sound-Induced Seizure.

Figure 4 illustrates the effect of RO 4-1284 on the EEG during sound stimulation. Spindle waves were prominent on all cortical leads after administration of RO 4-1284, but this EEG pattern was less obvious on the subcortical channels (not shown). Sound stimulation prompted an immediate disappearance of RO 4-1284 induced spindles. During the early tonic phase (Type D depicted in Figure 1) the amplitude of the EEG diminished and the frequency increased (Tracing A frequency increased from 10 to 21 Hz). As tonus continued, the amplitude of the EEG increased rapidly on all leads. Some of this high amplitude activity may be attributed to severe muscle contractions which occurred during the convulsion.

Electrostimulation of the IC in an AGS rat produced a displacement of the base line of the EEG tracing (Figure 5). Electrical stimulation of the IC was discontinued at the onset of the convulsion. During tonus all leads displayed a predominant frequency of 9 Hz. Spike activity in the cortex appeared to continue throughout the tonic phase of the convulsion.

Figure 6 shows the changes in electrical activity of the brain produced by stimulation of the MG. Large amplitude spikes appeared on cortical leads during electrical stimulation and continued after stimulation was terminated. Displacement of the base line of the subcortical leads during electrical stimulation prevented the recording of electrical changes in the RF and IC. No convulsive activity was observed during the period of spike activity on the EEG. Spike activity ceased abruptly during bursts of vigorous motor activity.

Figure 4. Electroencephalogram of a Partially Restrained Audiogenic Seizure Susceptible Rat After RO 4-1284, 5 mg/kg, during Sound-Induced Seizure.

Tracing A represents the voltage difference between the frontal cortical electrode and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing B represents the voltage difference between the parietal cortical electrode and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing C represents the voltage difference between the electrode implanted in the medial geniculate and the parietal cortical electrode at an amplification of 100 μv/cm.

Tracing D represents the voltage difference between the two tips of the bipolar electrode implanted in the medial geniculate at an amplification of 30 μv/cm.

Tracing E represents the voltage difference between the electrode implanted in the medial geniculate and the occipital cortical electrode at an amplification of 100 μv/cm.

Tracing F represents the voltage difference between the electrode implanted in the medial geniculate and the frontal cortical electrode at an amplification of 100 μv/cm.

The predominant frequency of a 2-second interval of the EEG tracing is noted directly beneath that interval in the figure.

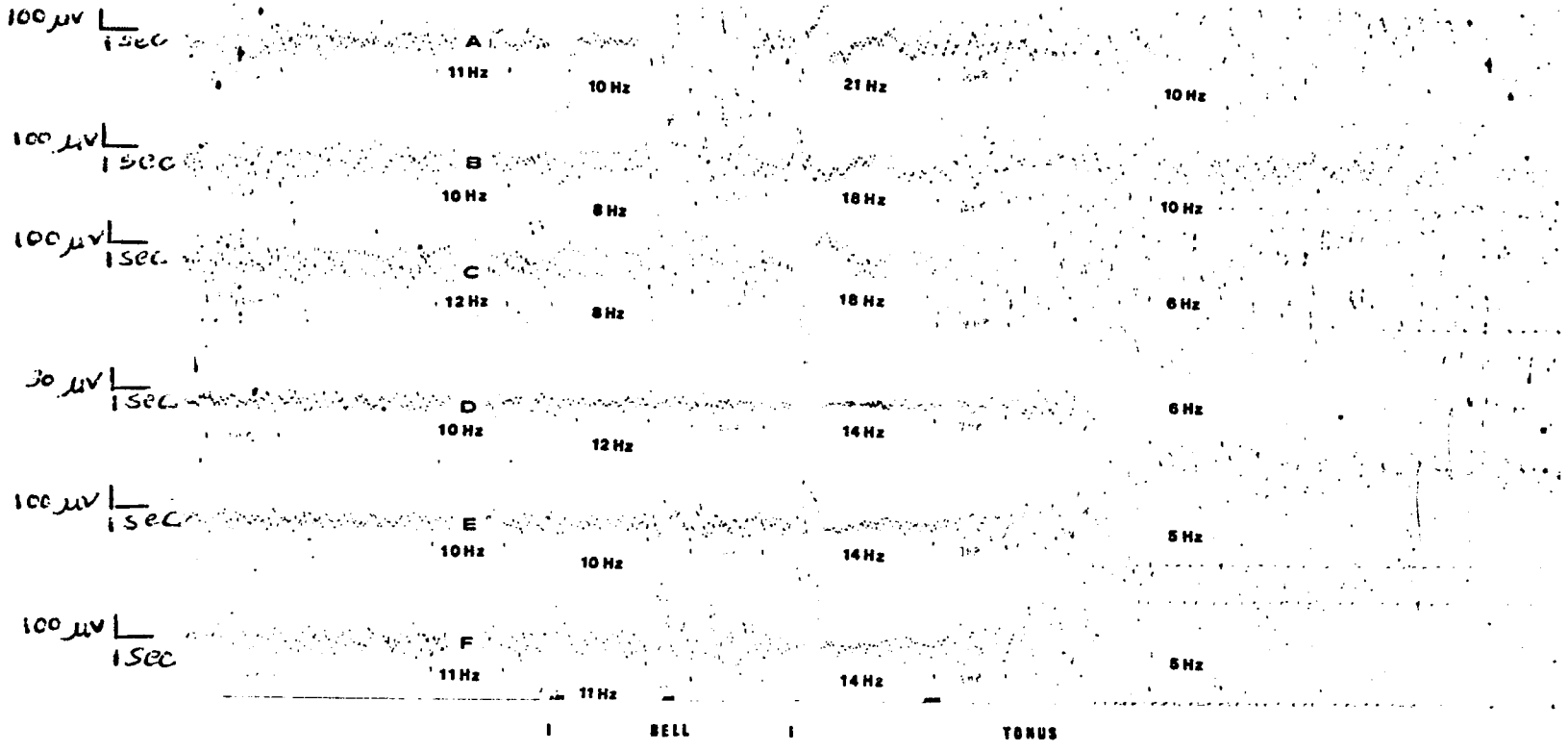


Figure 4. Electroencephalogram of a Partially Restrained Audiogenic Seizure Susceptible Rat After RO 4-1284, 5 mg/kg, During Sound-Induced Seizure.

Figure 5. Electroencephalogram During Seizure Induced by Electrical Stimulation of the Inferior Colliculus in an Audiogenic Seizure Susceptible Rat.

Tracing A represents the voltage difference between the occipital and parietal cortical electrodes at an amplification of 100 μv/cm.

Tracing B represents the voltage difference between the parietal and frontal cortical electrodes at an amplification of 100 μv/cm.

Tracing C represents the integrator response for Tracing B with an integrator sensitivity of 3.0 and a threshold of 0.8.

Tracing D represents the voltage difference between the two tips of the bipolar electrode implanted in the reticular formation at an amplification of 30 μv/cm.

Tracing E represents the voltage difference between the electrodes implanted in the reticular formation and the medial geniculate at an amplification of 30 μv/cm.

Tracing F represents the voltage difference between the two tips of the bipolar electrode implanted in the medial geniculate at an amplification of 30 μv/cm.

The predominant frequency of a 2-second interval of the EEG tracing is noted directly beneath that interval in the figure.

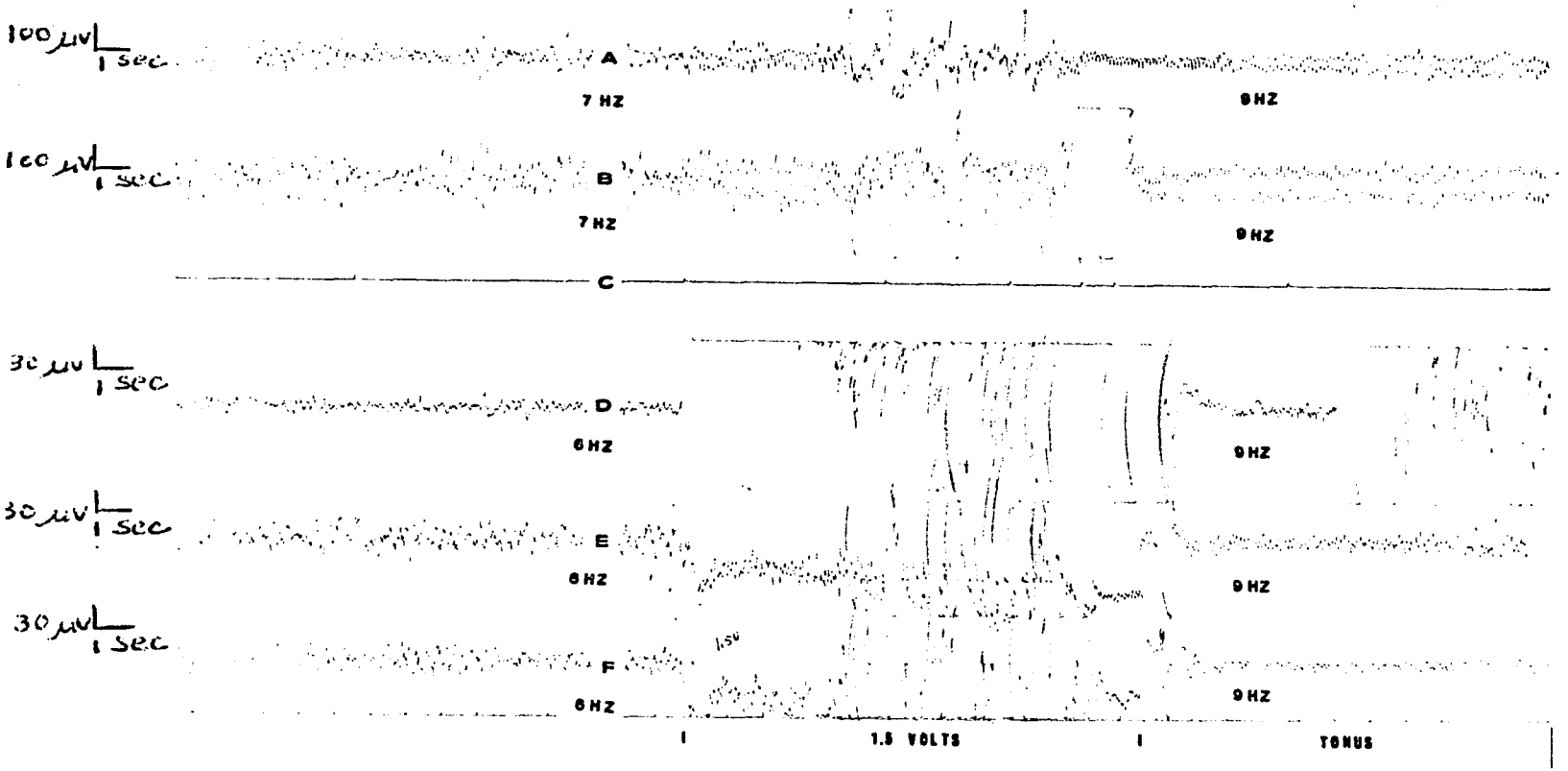


Figure 5. Electroencephalogram During Seizure Induced by Electrical Stimulation of the Inferior Colliculus in an Audiogenic Seizure Susceptible Rat.

Figure 6. Electroencephalogram During and Following Electrical Stimulation of the Medial Geniculate in Non-audiogenic Seizure Susceptible Rat.

Tracing A represents the voltage difference between the occipital and parietal cortical electrodes at an amplification of 100 μv/cm.

Tracing B represents the voltage difference between the parietal and frontal cortical electrodes at an amplification of 100 μv/cm.

Tracing C represents the integrator response for Tracing B with an integrator sensitivity of 3.0 and a threshold of 0.8.

Tracing D represents the voltage difference between the two tips of the bipolar electrode implanted in the reticular formation at an amplification of 30 μv/cm.

Tracing E represents the voltage difference between the electrodes implanted in the reticular formation and the inferior colliculus at an amplification of 50 μv/cm.

Tracing F represents the voltage difference between the two tips of the bipolar electrode implanted in the inferior colliculus at an amplification of 50 μv/cm.

The predominant frequency of a 2-second interval of the EEG tracing is noted directly beneath that interval in the figure.

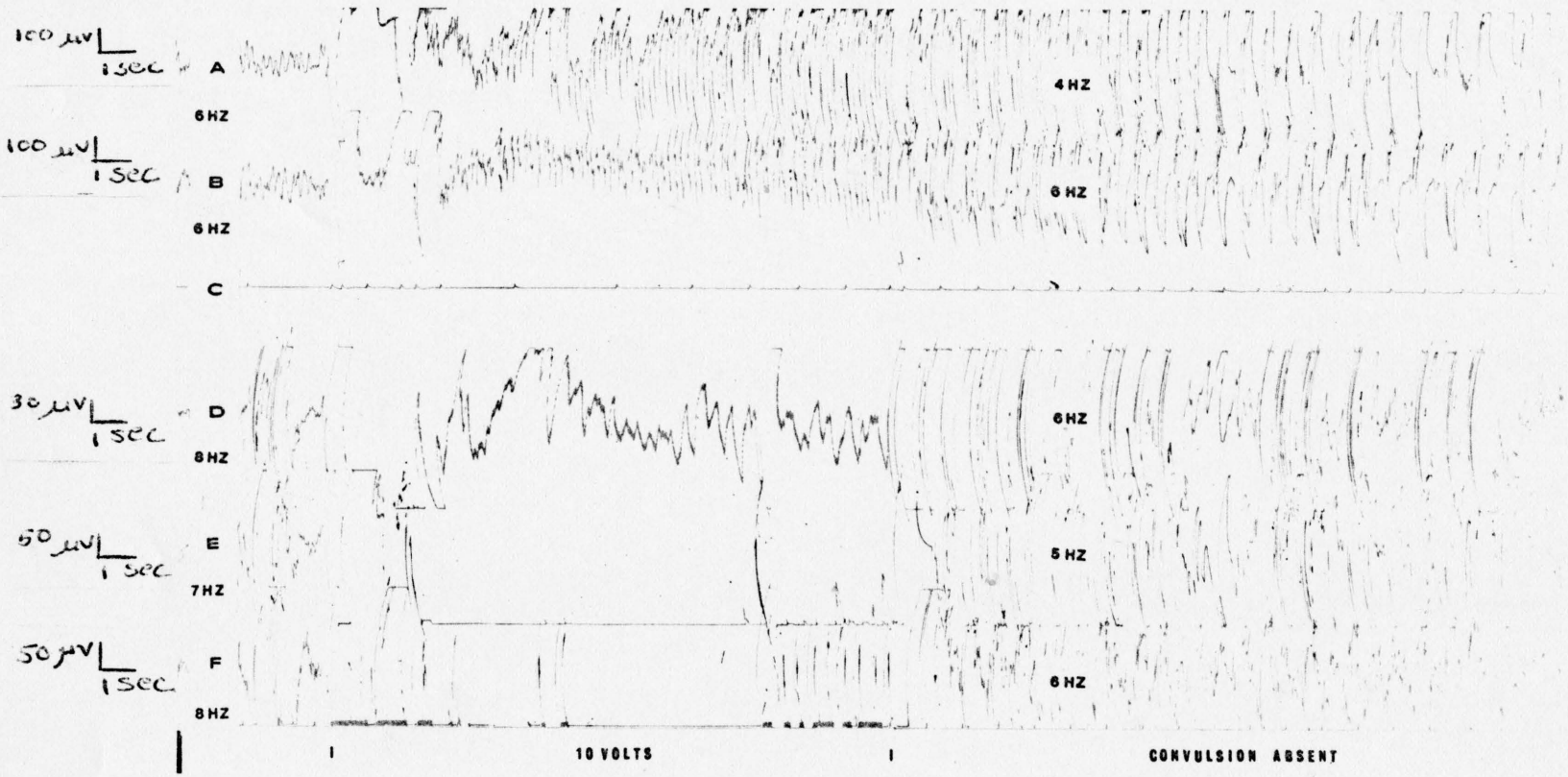


Figure 6. Electroencephalogram During and Following Electrical Stimulation of the Medial Geniculate in Non-audiogenic Seizure Susceptible Rat.

Discussion

A complete and permanent blockade of audiogenic seizures ensues after bilateral ablation of the inferior colliculus (IC), an observation which is consistent with the findings of Kesner (1966) and Wada et al. (1970). Lesions in the ventral cochlear nucleus (VCN) also produce loss of audiogenic seizure susceptibility, but this effect is temporary. The return of seizure susceptibility may be attributed to the incomplete destruction of the VCN as revealed by histological examination. Since ablation of other nuclei of the central auditory pathway fails to affect audiogenic seizure susceptibility, the VCN and IC emerge as the prime nuclei for further consideration.

The mechanism of audiogenically-induced seizures has not been elucidated. It may be hypothesized that AGS rats have one or more defects or anomalies in the auditory pathway such that auditory impulses spread massively beyond auditory neurons to cause running and convulsions. The most likely neurological structures through which such seizure activity may escape from the auditory system would be the auditory nuclei. The ventral cochlear nucleus is the structure in the CNS where auditory neurons form the first synapse. All sound input must pass through this structure in order to gain access to the remainder of the auditory pathway. The temporary refractoriness to audiogenic seizures following acute injury to the VCN may be due to temporary loss of auditory input to other parts of the auditory system which are responsible for allowing escape of seizure activity or due to temporary interference with the primary anomaly in the VCN which is responsible for susceptibility to sound-induced seizure. If the anomaly which allows auditory

impulses to spread outside of the auditory system resides in the VCN, it could be expected that ablation of a nucleus further up the auditory pathway would not block audiogenic seizure. Conversely, if the anomaly resides further up the auditory pathway rather than in the VCN, removal of the anomaly by ablation would be expected to block audiogenic seizures even if the VCN were left intact. In reality, it was found that bilateral ablation of the IC caused complete and irreversible loss of susceptibility to sound-induced seizures, despite the fact that no surgery was performed on the VCN. Hence, of the two auditory nuclei under consideration, the IC appears to be the more important with regard to seizure susceptibility. For this reason, subsequent studies were performed on the IC to evaluate further its probable role in the genesis of audiogenic seizures.

The contention that the IC is an important determinant of susceptibility to audiogenic seizure is supported by several observations. For example, electrostimulation of the IC in AGS rats results in a behavioral pattern of running and convulsion which is indistinguishable from that produced by sound stimulation. This pattern of behavior can also be produced in non-AGS rats through stimulation of the IC, although the convulsions are somewhat weaker. On the other hand, stimulation of other brain areas such as the RF or MG also produces convulsive activities, but the patterns of response do not resemble those produced by sound stimulation. Furthermore, the electroseizure threshold in the IC of AGS rats is some sevenfold lower than that in non-AGS rats. That this observation is not a reflection of a generally more sensitive nervous system in AGS rats is shown by the fact that there is no difference in

the electroseizure threshold of the RF and MG between AGS and non-AGS rats.

The observation that AGS rats have lower maximal and minimal electroshock seizure thresholds than non-AGS rats implies that they may have a lower level of inhibitory activity or a higher level of facilitory activity in certain areas of the CNS. Norepinephrine (NE) and 5-hydroxytryptamine (5-HT) have been implicated as inhibitory modulators in audiogenic seizure and they exist in lower concentrations in some regions of the brain of AGS rats than in non-AGS rats. Thus, the lower electroshock seizure thresholds in AGS rats may be ascribed to the relative deficit of these biogenic amines in certain parts of the brain. However, relative lack of NE and 5-HT cannot account for susceptibility to audiogenic seizure, since it has been demonstrated in this and other laboratories that depletion of NE and 5-HT in non-AGS rats will not render them susceptible to sound-induced seizure.

Audiogenic seizure susceptible rats appear to have at least two overt characteristics of the CNS that makes them distinctive from non-AGS rats. They can be made to convulse with sound stimulation and they have lower electroshock seizure thresholds. That the two characteristics are separate phenomena is demonstrated by the electroshock seizure studies involving the use of AGS rats before and after they were made refractory to sound-induced seizures. The observation that maximal and minimal electroshock seizure thresholds remain the same despite the development of refractoriness to audiogenic seizure provides supporting evidence for this concept.

The electroencephalographic studies were conducted to obtain information with regard to changes in brain electrical activity which occur during seizures initiated by sound and by electrical stimulation of specific brain structures. The violent motor activity which occurs during audiogenic seizure generates excessive electrical artifacts on the EEG which obscures the electrical changes originating in the brain. This technical difficulty diminishes the usefulness of the EEG in the study of audiogenic seizures. Complete physical restraint (with elastic bandage) or skeletal muscle paralysis (with d-tubocurarine) diminishes movement artifacts but also completely eliminates sound-induced running and convulsions. The EEG showed no evidence of seizure activity.

The observation that non-AGS rats have a higher maximal electroshock seizure threshold following sound treatment that induces refractoriness in AGS rats is unexpected. It could only be conjectured at this time that the daily repeated sound stimulations may induce CNS changes which exert anticonvulsive effects. For example, there may be increased inhibitory activity in certain regions of the brain. The reason that a similar change in electroshock seizure threshold was not induced in AGS rats may be related to the inability of certain deficient inhibitory systems (e.g., NE and 5-HT) to adjust to the stress of repeated sound stimulation.

It is suggested that proliferation of the audiogenic seizure pattern only requires sound stimulation to induce running activity. Once an AGS rat enters the running phase the sound stimulation can be termnated and the rat will continue to run until it convulses, in the case of a 1-run seizure pattern. In the case of the 2-run seizure pattern,

sound stimulation would have to be reinitiated following the pause and continued until the second running phase has started.

Extremely high amplitude seizure discharge in the cortex is not commonly seen even during the most severe audiogenic seizure. However, treatment of AGS rats with RO 4-1284, which depletes catecholamines and 5-HT, results in the generation of high amplitude cortical spikes during the ictal phase (Figure 4). This spike activity during the tonic-clonic phase of the seizure may be a manifestation of reduced resistance to spread of seizure activity in the cortex consequent to reduction of catecholamines and/or 5-HT inhibition. The effect of RO 4-1284 on EEG activity during audiogenic seizure correlates well with its ability to enhance the severity of convulsions.

The ictal portion of the EEG, which follows electrical stimulation of the IC, resembles the EEG observed during sound-induced convulsions. On the other hand, electroencephalograms recorded during stimulation of RF and MG display a dissimilarity to the electroencephalograms obtained from AGS rats during sound-induced seizures. These observations further emphasize the importance of the IC in the genesis of audiogenic seizures.

CHAPTER V

DEMONSTRATION OF A POTENTIAL REGULATORY ROLE OF γ-AMINOBUTYRIC ACID (GABA) IN THE INFERIOR COLLICULUS (IC) UPON SUSCEPTIBILITY TO AUDIOGENIC SEIZURE

Introduction

In a previous section (Chapter III) it was demonstrated that RO 4-1284, in a dose which increases audiogenic seizure severity failed to restore sensitivity to sound-induced seizure in refractory AGS rats. On the other hand, INH, in doses that increase audiogenic seizure severity, restored sensitivity to sound-induced seizure in refractory animals. Since RO 4-1284 reduces brain indoleamine and catecholamine concentrations, whereas INH reduces brain γ-aminobutyric acid (GABA) concentration, it is suggested that deficit of GABA may be a more important factor than deficit of the biogenic amines in audiogenic seizure susceptibility. This phase of the investigation will be devoted to consideration of GABA as a neurotransmitter involved in the determination of audiogenic seizure susceptibility.

Function of γ-Aminobutyric Acid

γ-Aminobutyric acid was introduced as a potential CNS neuromediator by Roberts and Frankel (1950) who reported that GABA exists in high concentrations in the CNS of animals. Evidence for an inhibitory neurotransmitter function for GABA in the stretch receptor system of crayfish is formidable (Kravitz, Kuffler, and Potter, 1963; Takeuchi

and Takeuchi, 1965; Otsuka, Kravitz, and Potter, 1967). In vertebrates, a neurotransmitter function in the CNS for GABA is strongly suggested (Baxter, 1970). A special function for GABA is implied by the high concentration of GABA which is found exclusively in the CNS and the irregular distribution of this amino acid throughout the CNS (Roberts and Kuriyama, 1968; Baxter, 1970). A remarkable correlation has been established recently between GABA concentration, GABA uptake, and GABA postsynaptic receptor distribution by Enna, Kuhar, and Snyder (1975). γ-Aminobutyric acid is released spontaneously from nervous tissue and this release can be accentuated by electrical stimulation (Okada and Hassler, 1973; Collins, 1974). Neuronal release of GABA is thought to be associated with synaptic inhibition (Mitchell and Srinivasan, 1969). In this regard, it has been shown that iontophoretic application of GABA mimics cortical synaptic inhibition (Krnjevic and Schwartz, 1967). Inhibitory systems which are suspected to utilize GABA as the mediator contain high concentrations of GABA. γ-Aminobutyric acid concentrations in the nerve terminals are estimated to be between 50 and 150 mM while GABA concentrations in Purkinje cell bodies are 6 mM (Fonnum and Walberg, 1973a and 1973b). Recent investigations of the CNS of vertebrates have revealed that GABA exerts an inhibitory effect on a variety of neurons, such as cortical neurons (Krnjevic and Schwartz, 1967), granule cells of the olfactory bulb (Nicoll, 1971), Deiter's neurons (Obata et al., 1967), and Purkinje cells (Roberts and Kuriyama, 1968).

Additional circumstantial evidence for a GABA neurotransmitter function is provided by the presence of transport and storage mechanisms for GABA in nervous tissue. Highly specific neuronal uptake mechanisms

have been demonstrated for GABA (Roberts and Kuriyama, 1968; Balcar and Johnston, 1973). Levi and Raiteri (1973) have detected a high and low affinity GABA transport system. These investigators propose that the low affinity system is associated with non-synaptosomal transport while the high affinity system is representative of synaptosomal uptake of the amino acid. Accumulation of GABA into synaptic vesicles has been demonstrated and this phenomenon provides an intraneuronal storage mechanism for GABA in presynaptic nerve terminals (Roberts and Kuriyama, 1968; Kuriyama, Roberts, and Kakefuda, 1968). Both neuronal and vesicular transport systems are sodium dependent (Roberts and Kuriyama, 1968) whereas sodium independent binding of GABA appears to represent postsynaptic receptor interaction (Zukin, Young, and Snyder, 1974; Enna et al., 1975).

γ-Aminobutyric acid appears to produce presynaptic and postsynaptic inhibition in the CNS. Presynaptic inhibition is achieved through a partial depolarization of the excitatory nerve terminal by GABA, which results in a diminished excitatory transmitter release, while hyperpolarization of the perikaryal membrane, produced by GABA, is responsible for postsynaptic inhibition. Both forms of GABA inhibition result from an increase in membrane conductance due to enhanced membrane permeability to chloride ion (Takeuchi and Takeuchi, 1967; Roberts and Kuriyama, 1968; Obata, 1972). All the evidence strongly favors the acceptance of GABA as an inhibitory neuromediator in the CNS.

Metabolism of γ-Aminobutyric Acid

γ-Aminobutyric acid is metabolized in the GABA bypass or shunt in the tricarboxylic acid cycle (Roberts and Kuriyama, 1968; Balazs et al., 1970). Glutamic acid, formed by transamination of α-ketoglutarate, is decarboxylated by the enzyme glutamic acid decarboxylase (GAD) to form GABA. The decarboxylation step is irreversible under physiological conditions.

γ-Aminobutyric acid is catabolized to succinic semialdehyde by GABA-α-ketoglutaric acid transaminase (GABA-T). No evidence exists to support the view that this step operates in reverse in vivo. Succinic semialdehyde reenters the tricarboxylic acid cycle as succinate, and this conversion is mediated by the enzyme, succinic semialdehyde dehydrogenase (SSA-D). Both GAD and GABA-T require the co-factor pyridoxal phosphate, whereas SSA-D utilizes NAD+ as its co-factor (Molinoff and Kravitz, 1968; Baxter, 1970; Balazs et al., 1970). Steady state GABA brain levels are controlled by GAD activity (Roberts and Kuriyama, 1968). Although GAD is inhibited by GABA in the lobster inhibitory neurons (Molinoff and Kravitz, 1968), this end-product feedback mechanism does not appear to function in the mouse brain (Baxter, 1970). Roberts and Kuriyama (1968) believe that GAD activity in mammals is regulated by chloride ion.

GABA may be inactivated in the synapse by uptake into the post-synaptic cell where it is rapidly destroyed by high intracellular levels of GABA-T, by uptake into neighboring glial cells where it is subsequently destroyed by GABA-T, and by uptake into the presynaptic nerve terminal where it is stored and reutilized as a neurotransmitter (Roberts

and Kuriyama, 1968; Henn and Hamberger, 1971; Watkins, 1972). Thus, brain GABA level, which is measurable, is an accumulation of active and inactive pools. This multifaceted compartmentalization of GABA in brain tissue complicates the evaluation of the role of GABA in seizure activity. Synaptic GABA concentration, which is not technologically feasible to determine, would be the most accurate correlate of GABA activity (Wood, 1975).

γ-Aminobutyric Acid Antagonists

Blockade of GABA synthesis by inhibition of GAD depletes endogenous stores of GABA and, thus, diminishes the inhibitory effect of GABA. For example, hydrazides, such as thiosemicarbazide and isoniazid (INH), decrease GABA by disrupting the activity of GAD. The main mechanism by which INH reduces GABA levels is indirect inhibition of GAD through depletion of pyridoxal phosphate by blockade of pyridoxal kinase. In addition, INH may possess a weak direct inhibitory effect on GAD. Since GAD possesses a weak affinity for pyridoxal phosphate, a reduction in this essential co-factor will create a selective inhibition of GAD and a fall in GABA levels (Baxter, 1970; Abrahams and Wood, 1970).

Blockade of pre- or postsynaptic receptors by non-activating molecules will prevent GABA from interacting with its receptors. An example of this type of GABA antagonist is bicuculline (BCC) which reversibly blocks GABA receptors involved in both presynaptic and postsynaptic inhibition (Bisti, Iosif, and Strata, 1971; Obata, 1972; Dreifuss and Matthews, 1972; Huffman and McFadin, 1972a and 1972b; Peck, Schaeffer, and Clark, 1973). Bicuculline does not interfere with neuronal uptake

or with enzymes of the GABA-shunt, GAD or GABA-T (Beart and Johnston, 1972).

γ-Aminobutyric Acid Distribution

Distribution of GABA in the CNS exhibits considerable parallelism among species. In the rabbit, rat, guinea pig, baboon, and Rhesus monkey the highest concentrations of GABA occur in the substantia nigra and globus pallidus. High levels of GABA are also found in the hypothalamus and the superior and inferior colliculi (Fahn and Cote, 1968; Okada et al., 1971).

γ-Aminobutyric Acid and Seizures

Although a variety of drugs are believed to cause convulsions by diminishing GABA inhibition in the CNS, the degree of changes in brain GABA concentration induced by these drugs do not appear to correlate consistently with the degree or incidence of seizure response. The wide range of changes in brain concentration of GABA which is related to seizure induced by these drugs may be due to different degrees of depletion of nonfunctional pools of GABA, despite consistent depletion of functional pools of this neurotransmitter. Another correlation that has been reported is decrease in GAD activity observed at the onset of convulsions induced by GABA depleting drugs. However, the percent decrease in GAD activity also varies greatly from drug to drug (Wood, 1975). Because of the difficulty in attempting to draw a simple relationship between GABA levels and onset of convulsions, Wood (1975) devised an equation to predict seizure activity based on GAD activity and GABA concentration and suggested that brain excitability is inversely related to

these factors. Cobalt induced epilepsy in animals provides additional evidence that diminished GAD and/or GABA may be involved in causing seizures. Emson and Joseph (1975) reported that GAD activity and GABA concentraton are reduced at the primary lesion produced by cobalt and that GAD activity in the secondary or mirror focus is significantly lowered as well.

If GABA were to function in seizures as an inhibitory neurotransmitter such that its depletion results in the appearance or worsening of convulsions, it would be expected that increasing its concentration in the brain would attenuate or prevent seizures. Indeed, it is reported that agents that inhibit GABA-T, such as n-dipropyl-acetate (Simler et al., 1973), 2-methyl-2-ethyl caproic acid or 2,2-dimethyl valeric acid (Maitre, Ciesielski, and Mandel, 1974), block audiogenic seizures during the period when they cause elevation of brain GABA concentration.

It has been demonstrated in a previous section (Chapter IV) that the IC is an important part of the auditory system concerned with the genesis of audiogenic seizure. In view of the fact that GABA is normally found in high concentration in the IC and in view of the observations that reducing brain concentration of GABA results in convulsions, it is hypothesized that the anomaly in the IC which is responsible for susceptibility to audiogenic seizures is a deficit of GABA.

The objective of this phase of the investigation is to evaluate the IC for evidence of GABA deficiency by bilateral injections of GABA and/or bicuculline.

Method

Solutions of drugs were injected into the IC of the rat brain at a constant volume of 0.5 microliters per IC in order to assure consistent localization of drug effect (Myers, 1966; Routtenberg, 1972). To evaluate the effect of GABA on audiogenic seizures, the amino acid was injected bilaterally into the IC. Five minutes after injection the animal was exposed to sound stimulation or injected bilaterally in the IC with bicuculline (BCC) and the behavioral response was noted. AGS rats were used and GABA was injected into the IC of each animal under restraints outside the test chamber. The injection needles and tubing used for the administration of GABA were removed and replaced with needles and tubings which contained BCC. The animal was placed in the behavioral chamber and BCC was administered five minutes after GABA. The rat was observed for fifteen minutes for seizure activity. Graded doses of BCC were injected bilaterally into the IC of both AGS and non-AGS rats. Behavioral changes induced by these injections were then observed and recorded.

Results

Bilateral injections of GABA into the IC of AGS rats in graded doses produced dose related blockade of seizures induced by sound and of seizures induced by bilateral injections of bicuculline into the IC five minutes following GABA administration (Table 14). The localized injections of GABA in the brain produced no spontaneous behavioral changes. The paired injections of BCC into the IC of AGS rats elicited a seizure pattern identical with that produced by sound. Within 5 to 10 seconds following injections of BCC, the rats began a vigorous running phase

Table 14. Anticonvulsant Effect of γ-Aminobutyric Acid (GABA) Administered Bilaterally into Inferior Colliculi (IC) of Audiogenic Seizure Susceptible Rats.

Convulsive Stimulus	Percent Rats Protected from Seizures					
	Saline	GABA, μMOLES/0.5 μl/IC				
		0.157	0.312	0.625	1.25	2.50
Sound (Doorbell 110 db)	0 (7)[a]	--	--	28.6 (7)	57.1 (7)	100 (7)
Bicuculline (0.04%, 0.5 μl/IC)	0 (7)	16.7 (6)	71.4 (7)	83.3 (6)	87.5 (6)	100 (6)

a. Number of rats tested.

which terminated in a full motor seizure. The intensity of the response induced by BCC ranged from a marginal tonic convulsion to a full tonic hind limb extension convulsion (i.e., from Type A to Type D convulsions depicted in Figure 1). The severity of the BCC induced seizure was reproducible for each animal and was generally consistent with the seizure intensity elicited by sound for each animal. Following the convulsion a "furor" or "rage" phase of high intensity commonly occurred which is succeeded by depression. Only one seizure episode occurred following one set of bilateral BCC injections. The elapsed time for the seizure episode, from completion of the injections to onset of postictal depression, is under 120 seconds. Successive bilateral injections of BCC during the post convulsive depression phase provoked successive convulsions, with the same seizure pattern.

Bilateral injections of BCC in graded doses into the IC of AGS and non-AGS rats produced dose related responses in both types of animals (Table 15). The major difference in the responses of the two types of animals is that the AGS rats exhibited a very steep dose response at the lower doses, whereas the non-AGS rats exhibited a more gradual dose response which involved the higher doses of BCC. In addition, the severity of convulsions in non-AGS animals did not exceed marginal tonic convulsions (Type A as depicted in Figure 1).

Discussion

Localized injections of GABA into the IC, a structure demonstrated to be important in audiogenic seizure susceptibility, blocks audiogenic seizures without causing symptoms of overt behavioral changes. These

Table 15. Convulsive Response Evoked by Bilateral Administration of Bicuculline into the Inferior Colliculi of Audiogenic and Non-audiogenic Seizure Susceptible Rats.

Rat Type	Number of Rats Convulsed/Number of Rats Injected					
	Saline	Bicuculline Concentration (%)[a]				
		0.03	0.04	0.10	0.14	0.18
Audiogenic	0/11	0/4	9/9	2/2	--	--
Non-audiogenic	0/8	--	0/8	2/8	5/8	6/8

a. A volume of 0.5 µl of drug solution was injected into each inferior colliculus sequentially. A 10 second interval was required to complete each pair of injections.

results are in distinction to those previously observed in this laboratory, in which intraventricular injections of GABA also protected against audiogenic seizures, but the animals showed generalized depression, ataxia, and motor paralysis. Because intraventricular injection produces a general CNS distribution of exogenous GABA in the earlier study and because of the depressant effects, the anticonvulsant effect may be ascribed to a non-selective CNS depression. On the other hand, because of a rather selective response, it is suggested that injections of GABA into the IC of AGS rats represent repair of a deficiency in the IC and reflect the functional role of endogenous GABA in the IC and other auditory nuclei, namely inhibition and moderation of auditory impulses to restrict them to the auditory pathway. Indeed, other investigators have reported that high concentrations of GABA are normally found in the IC (Fahn and Cote, 1968; Okada et al., 1971) and that GABA has an inhibitory role in the VCN (Watanabe, 1971) as well as in the IC (Watanabe and Simada, 1971).

Injection of constant doses of bicuculline (BCC) into the IC of AGS rats (0.5 µl/IC of a 0.04% solution) consistently produces a convulsive seizure pattern that resembles the pattern of sound-induced seizures. Prior treatment of the IC with graded doses of GABA elicited a dose related reduction of BCC-induced convulsions. This dose related antagonism in the IC provides additional evidence that GABA may play an inhibitory role in the IC to restrain auditory impulses. That the interaction of GABA and BCC are indeed localized in the IC is suggested by the observations of Myers (1966) that 0.5 µl volumes of solutions injected into brain tissue display a spherical diffusion pattern about the cannula tip

with an average spread of 1 mm, as compared to the dimensions of the IC in the rat which is 4 x 3.5 x 3 mm.

Finally, AGS rats require less BCC injected into the IC than non-AGS rats to produce seizures. This observation further suggests that the AGS rats have less GABA-mediated inhibition in the IC.

CHAPTER VI

GENERAL DISCUSSION

Epilepsy is a complex and incompletely understood neurological disorder. Only a limited amount of experimental data on this convulsive disease is obtainable directly from human studies. Many of the fundamental concepts of epilepsy have been derived from studies in animals. Various animal models have been employed although few models bear appreciable resemblance to the clinical disease. Reflex seizures in animals afford excellent opportunities to study certain phenomena which may be comparable to those associated with epilepsy in man. For example, in audiogenic seizure susceptible (AGS) animals, sound initiates seizures which are reminiscent of "cursive" epilepsy, a form of grand mal epilepsy which is characterized by extreme fear followed by frantic running, tonic convulsion, clonic convulsion and postictal depression (Geist, 1962, p. 17).

The seizure response of AGS animals is influenced by the nature of the sound to which they are exposed. For example, Alexander and Gray (1970) reported that pure tone between 5 and 35 KHz is highly effective for producing seizure in AGS mice. In comparison, the present study revealed that electric doorbells are most effective in producing seizures in AGS rats. Pure tone and mixed frequency sound provided by a random noise generator are completely ineffective. The sound of an air jet and high fidelity tape recordings of electric doorbells are both moderately

effective, producing seizure in about 45% of AGS rats tested, but the latency period is several-fold longer than that for electric doorbells. Although electric doorbells have been routinely employed in this laboratory, the present observations represent the first comparative evaluation of various sounds which may be effective in producing convulsive seizures through auditory stimulation. The results of this study serve to validate use of electric doorbells in audiogenic seizure studies in rats.

Another factor which has been of concern in audiogenic seizure research is the effect of repeated sound stimulation on responsiveness to audiogenic seizures. It was found that a single sound stimulation per week for 5 weeks produced no significant change in seizure response, whereas a single stimulation per day produced progressively poorer responses over several weeks. On the other hand, 10 or 20 closely repeated sound stimulations per day caused a high percentage of AGS rats to become refractory to audiogenic seizure within several days. This refractory state persists for several days and can be maintained for long periods by subsequent single daily sound stimulations. These observations agree with the report of Daliers and Rigaux-Motquin (1968) and confirm the belief that susceptibility to sound-induced seizures can be suppressed by repeated sound stimulations. Hence, it is important in research involving use of AGS animals that testing of these subjects be properly spaced.

The mechanism of sound-induced seizures has not been elucidated. It is hypothesized that AGS rats have one or more defects in the auditory pathway, possibly in central auditory nuclei, which allow auditory

impulses to spread beyond auditory neurones to cause running and convulsions. Some investigators have shown that ablation of the inferior coliculus (IC) protects against audiogenic seizures (Kesner, 1966; Wada et al., 1970). Others have shown that neurones in the cochlear nucleus and the IC of mice "primed" for audiogenic seizures display exaggerated evoked potentials to auditory input (Saunders et al., 1972). In this laboratory it was demonstrated that bilateral lesions of the ventral cochlear nucleus (VCN) or the IC blocked audiogenic seizures, but that ablation of the superior olivary complex (SOC) or the medial geniculate (MG) did not. If the defect resides in the VCN it would be expected that ablation of the VCN would block audiogenic seizures but ablation further up the auditory pathway would not. If the defect does not reside in the VCN, but further up the auditory pathway, it would be expected that ablation of the defect upstream from the VCN would block audiogenic seizure. Analogous rationalizations can be made with regard to other auditory nuclei further up the auditory pathway. Thus, the concept is advanced that a defect in the IC is responsible for susceptibility to audiogenic seizure. Additional support for this contention include the observation that electrical stimulation of the IC of AGS or non-AGS rats produces running and convulsions, a pattern of response seen in audiogenic seizure, but this response pattern is not seen when other regions of the brain such as the MG or reticular formation (RF) are stimulated to produce convulsions. Furthermore, the electroseizure threshold in the IC of AGS rats is some sevenfold lower than that in non-AGS rats. This lower threshold is not a reflection of a generally more sensitive CNS in AGS

rats, since the electroseizure thresholds of the MG and RF in AGS rats are no different than those in non-AGS rats.

The observation that AGS rats have lower maximal and minimal electroshock thresholds than non-AGS rats relate well with previous findings that the inhibitory neurotransmitters norepinephrine (NE) and 5-hydroxytryptamine (5-HT) exist in lower concentrations in some regions of the brain of AGS rats than in non-AGS rats. Although relative lack of these amines may account for the lower electroshock seizure thresholds, it cannot account for susceptibility to audiogenic seizures. Relative sensitivity to general electroshock seizure and susceptibility to audiogenic seizure are apparently two distinct phenomena, since electroshock seizure thresholds are unchanged despite the development of refractoriness to audiogenic seizures.

With regard to the refractory AGS rats, it is important to note that the refractoriness to audiogenic seizure is reversed by the γ-aminobutyric acid (GABA)-depleting drug isoniazid, but not by the catecholamine (CA)-and 5-HT-depleting drug RO 4-1284. Similarly, it has been noted that non-AGS animals are not converted to AGS animals when brain CA and 5-HT are depleted (Lehman and Busnel, 1963; Jobe et al., 1973), but depletion of GABA increases audiogenic seizure susceptibility (Benson and Salzburg, 1966). These observations lend support to the belief that deficit of GABA is responsible for susceptibility to audiogenic seizures and provide impetus for additional studies.

Since the IC is implicated as the auditory nucleus most likely to be involved in susceptibility to audiogenic seizure it was further evaluated as a site wherein GABA might act. Bilateral injection of graded

doses of bicuculline (BCC), a GABA antagonist, into the IC of AGS and non-AGS rats produces a seizure pattern identical to an audiogenic seizure. The response is dose-related and occurs within 2 minutes following injection. The most notable difference in response between the two types of animals is that the AGS rats exhibit a very steep dose response at the lower dose range, whereas the non-AGS rats exhibit a more gradual dose response at the higher dose range. Graded doses of GABA, injected bilaterally into the IC five minutes before testing, produce dose-related blockade of seizures induced by sound or by bilateral injection of BCC into the IC. Because AGS rats are more sensitive to injections of BCC into the IC than non-AGS rats, it is suggested that they have a deficit of GABA in this auditory nucleus and therefore have less inhibitory activity in the IC than non-AGS rats. It is further suggested that the anti-audiogenic seizure effect of GABA injection in the IC represents temporary repair of a deficiency in the IC of AGS rats and may reflect the modulatory role of endogenous GABA in this and possibly other auditory nuclei.

The results of this investigation provide evidence that a defect in the GABA inhibitory system in the IC is an important factor responsible for susceptibility to audiogenic seizure. Thus, because of inadequate containment of impulses within the auditory pathway, sound stimulation results in spread of discharges from the IC which causes detonation of adjacent neurones and projection of seizure discharge to other parts of the central nervous system.

APPENDIX A

SURGICAL IMPLANTATION OF ELECTRODES

I. Equipment

A. Stereotaxic Equipment: David Kopf Instruments

B. Dental Drill: Model #73, Foredom Electric Co., Inc.

C. Doriot Hand Piece: Model #42, Foredome Electric Co., Inc.

D. Dental Burr: Number 700 carbide, S. S. White Co.
Number 4 Burr, Ramson and Randolph

E. Cortical electrode: A 1/8 inch stainless steel machine screw (H. M. Harper, Size 2-56) formed the major portion of the cortical electrode. A small portion of the screw head was filed to produce a flat surface to which a short segment of Teflon coated wire was soldered. Five millimeters of Teflon coating was stripped from the unattached end of the wire. The exposed wire was fashioned into a loop which was inserted into a copper male pin and soldered into position.

F. Bipolar Electrodes: A 60 centimeter segment of Teflon coated wire was folded into a loop and the cut ends were gripped in a vice. A wooden probe was inserted into the loop and the wire pulled taut. The probe was rotated clockwise until a tight spiral was formed over the whole length. The coiled wire was cut into short segments of appropriate length for the depth of implantation. One end of the segment was partially unraveled. Approximately 5 millimeters of coating was removed from the ends. The two bare wires were folded into loops and inserted into male copper pins and soldered firmly in place.

II. Materials

A. Pentobarbital: Nembutal, Abbott Labs

B. Orthodontic Resin: L. D. Caulk Co.

C. Duracillin A.S.: Eli Lilly and Co.

D. Surgical Thread: Twisted black silk, Size 000, Curity

E. Teflon coated wire: Stainless steel wire with coated diameter equal to 0.013 inches, Medwire Corp.

F. Copper male pins: No. 220-P02, Amphenol

G. Female plug: No. 223-1209, Amphenol

H. Gelfoam: Upjohn

III. Surgery

A. A 280 to 320 gram rat was anesthetized with pentobarbital, 30 mg/kg, IP.

B. The hair was clipped from the dorsal surface of head.

C. A midsaggital incision was made from the eyes to the back of the skull.

D. The skin was retracted to provide ample working area.

E. The periosteum was removed from the exposed skull by scraping.

F. The muscles were retracted from lateral portions of parietal bones along the parasagittal ridge.

G. Holes were drilled in the parietal bone on both sides of skull just beneath the parasagittal ridge.

H. A stainless steel machine screw (Size 2-56, 1/8 inch) was inserted in each hole (Anchor screw).

I. A hole was drilled through the skull at a point 2.0 mm lateral and 3.0 mm rostral to the bregma in the right frontal bone.

J. A second hole was drilled in the left parietal bone, 2.5 mm lateral and 1.5 mm caudal to the bregma.

K. A third hole was centered in the interparietal bone.

L. Cortical electrode screws were inserted into the 3 holes described in steps III-I, III-J, and III-K.

M. Surgical thread was tied to the right anchor screw and tightly wrapped around each adjacent screw in a clockwise fashion about the head. When the circuit was completed the terminal end was securely fastened to the right anchor screw.

N. The positions of the bipolar electrodes, determined stereotaxically, were marked on the skull with a felt tipped

marker. The coordinates were determined from the atlas of Pellegrino and Cushman (1967) and were as follows:

Medial Geniculate:	rostral-caudal:	2.0
	medial-lateral:	3.7
	dorsal-ventral:	6.7
Reticular Formation:	rostral-caudal:	0.4
	medial-lateral:	2.0
	dorsal-ventral:	6.5
Inferior Colliculi:	rostral-caudal:	-1.2
	medial-lateral:	1.7
	dorsal-ventral:	3.6

O. Three holes were drilled at the positions indicated for the bipolar electrodes and a sharp 26 gauge hypodermic needle was carefully inserted through the dura mater.

P. The bipolar electrode was lowered to the proper depth. The hole was enlarged if necessary for proper clearance. Gelfoam was packed around the electrode to close the opening in the skull. The electrode was fixed in place with a small amount of orthodontic resin. The electrode was removed from the carrier when the resin was firm. Each electrode was implanted in a similar manner until all were in place.

Q. All screws and electrodes were covered with a layer of orthodontic resin which was allowed to harden.

R. Male pine which were attached to the electrode wires were inserted into the female amphenol plug.

S. Wires were folded under the plug and all wires were completely embedded in orthodontic resin. The plug was pressed firmly against the skull and held until resin hardened. The sides of the resin "cap" were shaped into a smooth surface as the resin hardened.

T. Both ends of the incision were sutured until the skin rested firmly against the resin "cap."

U. Duracillin A.S., 0.3 m., was injected intramuscularly.

V. Animals were housed individually following surgery.

W. At least 14 days were allowed for recovery from surgery.

APPENDIX B

IMPLANTATION OF CANNULA

I. Equipment

A. Stereotaxic equipment: David Kopf Instruments

B. Dental Drill: Model No. 73, Foredom Electric Co., Inc.

C. Doriot Hand Piece: Model #43, Foredom Electric Co., Inc.

D. Dental Burr: Number 700 carbide, S. S. White Co.
Number 4 Burr, Ramsom and Randolph

E. Cannula: The cannula guide shaft was fashioned from a 23 gauge by 1 inch Monoject 200 disposable hypodermic needle. The needle was stainless steel while the hub was aluminum. The hub and shaft were cut with wire cutters and filed to an overall length of 12 mm. An inner stainless steel shaft remained within the guide cannula in order to maintain an open passage and was removed only at the time of insertion of the injection needle. The inner shaft was cut from a 30 gauge, short Monoject 400 dental needle. The length of the shaft was 12 mm. The aluminum hub was severed at the base of the plastic component with plier-type wire cutters. The cutting procedure closed the lumen of the shaft.

F. Injection needle: A monoject 400, 30 gauge, short, dental needle served as an injection needle. The shaft was clipped with wire cutters 13 mm from the base of the hub and was filed to approximately 12.5 mm so that the injection needle tip protruded about 0.5 mm from the cannula guide shaft. The plastic fitting on the hub was removed with the wire cutters. The shaft, which extended through the hub of the needle was inserted into a polyethylene tube (PE 20). The end of the tube was stretched to reduce the diameter of the lumen in order to insure a tight fit. The opposite end of the tube was attached to a 5 or 10 microliter syringe (Hamilton Co., Inc.).

G. Cannulae Holder: This holder was designed to insert two cannulae simultaneously into bilateral structures. Two 30 gauge Monoject 400 dental needles were prepared as described in section I-E for the inner cannula shaft except the shaft length was adjusted to 8 mm. The hubs of these two needles

were imbedded in orthodontic resin with the shafts extending in parallel at a distance of 3.4 mm apart. A rigid metal rod was centered between the 2 hubs and extended outward at a 180 degree angle to the shafts. The point of contact between the three components was solidly enclosed in orthodontic resin. The holder shafts were inserted into the lumen of the guide cannulae and the distance between the cannulae tips adjusted to 3.4 mm. A slight bend in the shafts of the holder held the guide cannulae firmly in place. The heavy metal rod on the opposite end was inserted into the stereotaxic carrier.

II. Materials

A. Pentobarbital: Nembutal, Abbotts Labs

B. Orthodontic Resin: L. D. Caulk Co.

C. Duracillin A.S.: Eli Lilly and Co.

D. Gelfoam: Upjohn

III. Surgery

A. A 280 to 320 gram rat was anesthetized with pentobarbital, 30 mg/kg, IP.

B. The hair was clipped from dorsal surface of head.

C. A midsaggital incision was made from the eyes to the back of the skull.

D. The skin was retracted to provide ample working area.

E. The pericsteum was removed from exposed skull by scraping.

F. Three holes were drilled through the skull. One was placed in the right frontal bone 2.0 mm lateral and 3.0 mm rostral to the bregma. The second hole was placed in the left parietal bone 2.5 mm lateral and 1.5 mm caudal to the bregma. The third hole was centered in the interparietal bone.

G. Stainless steel machine screws (Size 2-56, 1/8 inch) were placed in each hole.

H. Using stereotaxic coordinates the points of entry of the cannulae were marked on the skull:

Inferior Colliculi:	rostral-caudal:	-1.7
	medial-lateral:	1.6
	dorsal ventral:	4.0

I. Two holes were drilled through the skull at the points determined in step III-H. A sharp 26 gauge hypodermic needle was carefully inserted to penetrate the dura mater.

J. The guide cannulae was lowered to the proper depth. The openings were enlarged if necessary for proper clearances. Gelfoam was packed around cannulae to close openings in the skull.

K. The guide cannulae were enclosed in orthodontic resin and allowed to harden. The cannulæ holder was released from stereotaxic carrier. All screws were covered with resin and a resin "cap" was molded to slightly below the top of the hub of the guide cannulae. When the resin hardened, the cannulae holder was carefully removed.

L. A circular well was constructed around the hubs of the guide cannulae. This well prevented the animal from removing the inner steel shafts.

M. The inner steel shafts were inserted.

N. Both ends of the incision was sutured until the skin was firm against the resin cap.

O. Duracillin A.S., 0.3 ml, was injected intramuscularly.

P. Animals were housed individually following surgery.

Q. At least 14 days were allowed for recovery.

APPENDIX C

ELECTROLYTIC LESIONS

I. Equipment

A. Lesion Maker LM-4: Grass Instrument Co.

B. Stereotaxic Equipment: David Kopf Instruments

C. Dental Drill: Model #73, Foredom Electric Co., Inc.

D. Dariot Hand Piece: Model #42, Foredom Electric Co., Inc.

E. Dental Burr: No. 700 Carbide, S. S. White Co.
No. 4 Burr, Ramson and Randolph

F. Lesion Electrode: A 20 gauge stainless steel Howmet wire overlaid with seven coats of insulating resin formed this electrode. The steel wire was wiped clean with acetone and dipped into the insulating resin from which it was gradually withdrawn to prevent beading. The resin was dried overnight in a 90°C oven after each applied coat. Approximately 1 mm of resin was removed from the tip of the electrode. The electrode was examined for improper coating by submersion into egg white and application of full instrument voltage. Coagulation of protein occurred only at the exposed tip, which indicated complete insulation of the electrode shaft.

II. Materials

A. Pentobarbital: Nembutal, Abbott Labs

B. Duracillin A.S.: Eli Lilly and Co.

C. Gelfoam: Upjohn

D. Insulating Resin: Epoxylite #6001-M, Epoxylite Corp. of N.Y.

III. Surgery

A. A 280 to 320 gram rat was anesthetized with pentobarbital, 30 mg/kg, IP.

B. The hair was clipped from dorsal surface of head.

C. A midsaggital incision was made from the eyes to the back of the skull.

D. The skin was retracted.

E. The periosteum was removed from the skull by scraping.

F. Using stereotaxic coordinates the skull was marked for points of entry of electrodes.

G. The holes were drilled through the skull at points indicated in step III-F.

H. The dura mater was perforated with a sharp 26 gauge hypodermic needle.

I. The lesion electrode was inserted to proper depth.

J. A metal grounding plate was placed beneath the animal and saline was applied to improve electrical conduction.

K. Voltage was applied; voltage and duration of exposure were previously determined experimentally.

L. The electrode was slowly withdrawn and the opening in the skull was packed with Gelfoam.

M. The entire incision was sutured.

N. Duracillin A.S., 0.3 ml, was injected IM.

O. Animals were housed individually following surgery.

P. Testing began 24 hours after surgery.

APPENDIX D

HISTOLOGICAL TECHNIQUES

I. Equipment

A. Cryostat-Microtome: American Optical

B. Microscope: Bausch and Lomb Optical Co.

C. Guillotine: Harvard Apparatus Co., Inc.

D. Slide Warmer: Chicago Surgical and Electrical Co.

II. Materials

A. Formaldehyde: Mallinckrodt Chemical Works

B. Sodium Chloride USP: Mallinckrodt Chemical Works

C. Ether A.R.: Mallinckrodt Chemical Works

D. Egg Albumin

E. Glass Slides 3" x 1": Gold Seal, Clay-Adams

F. Cover slips: Corning Cover Glass No. 2,22 mm sq.

G. Ethanol 100%: U. S. Industrial Chemical Co.

H. Entellan: Rapid Embedding agent, E Merck Ag Dormstaat

I. Embedding Solution:

Sucrose -- 100 grams
Gum Acacia -- 5 grams
Distilled Water -- qs 500 ml
Small crystal of Thymol

The mixture of sucrose, acacia, and water was heated with constant stirring until dissolved and then diluted with distilled water to volume. A small crystal of thymol was added to serve as a preservative.

J. Cresyl Stain:

Cresyl violet acetate -- 1 gram
Distilled water qs -- 1000 ml.

III. Excision and Fixation of Tissue

A. The rat was anesthetized by ether inhalation.

B. The abdominal and thoracic cavities were exposed through a ventral midline incision.

C. 20 ml of normal saline was injected into the left ventricle of the heart with a 25 ml glass syringe and 18 gauge x 1 inch disposable needle.

D. The inferior vena cava was severed and the blood allowed to drain.

E. The animal was perfused with an additional 30 ml of normal saline.

F. Inferior vena cava was occluded with a hemostat and 20 ml of a 10% solution of formaldehyde in saline was injected in the left ventricle.

G. The hemostat from the vena cava was removed and the animal was perfused with an additional 30 ml of 10% formaldehyde.

H. The animal was decapitated and the brain removed.

I. The tissue was allowed to harden by storage in a 10% formaldehyde solution for at least 2 days.

IV. Serial Sections

A. Formaldehyde was washed from tissue by rinsing with a continuous stream of distilled water for 8 hours.

B. Tissue was soaked in embedding solution for 12 hours.

C. A block of tissue was cut from the appropriate brain region in the plane of the Atlas. See Richer (1967) for the procedure.

D. The block of tissue was frozen on the chuck in the cryostat by layering water at interface between tissue and metal.

E. Tissue was stored in cryostat for at least 30 minutes before slicing in order to thoroughly freeze tissue. Initial cryostat temperature of -8°C was adjusted as required for proper slicing.

F. The tissue was mounted in the microtome in order to produce sections in the same plane as those in the Pellegrino and Cushman (1967) Atlas. Section thickness was 40 micra.

G. Only periodic sections were retained for position determination and verification of plane orientation until the electrode or cannulae tracts appeared. Every fifth section was then retained until all signs of abnormal tissue disappeared.

H. Sections were placed on a glass side (1" x 3"), three per slide, previously coated with a thin layer of egg albumin.

I. Slides were dried on a 40°C slide warmer and stored in a 50°C oven overnight.

V. Staining

A. Slides were placed in a distilled water bath for 3 minutes.

B. Slides were transferred to cresyl stain bath at 50°C for one minute.

C. Slides were rinsed in distilled water for ten minutes.

D. Slides were transferred through a series of three alcohol baths of 70%, 95%, and 100%, respectively, allowing three minutes exposure in each alcohol bath.

E. The slides were then placed in two successive dishes of Xylene for three minutes each.

F. Each slide was covered with Entallan and a cover glass.

VI. Placement determination

A. Each section was examined under a microscope. Sections were matched with the comparable section in the Atlas to determine rostral-caudal coordinate (deGroot System).

B. The section was selected which was central to the tissue damage in the rostral-caudal plane.

C. Medial-lateral and dorsal-ventral coordinates were determined by comparison with the Atlas. Depth of placement was expressed in terms of distance from the dura rather than from stereotaxic zero.

D. The extent of the damage, especially to important neighboring structures, was observed.

APPENDIX E

STATISTICAL PROCEDURES

I. Student's *t* test

All calculations were performed according to the following formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{[S_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)]^{1/2}}$$

where:

t = calculated Student's *t* value

$\bar{X}$ = the mean value of a treatment

S_p^2 = the pooled variance

n = the number of experimental subjects per treatment

1 and 2 = the particular treatments under analysis.

The standard error of the mean (S.E.M.) was calculated by the following formula:

$$\text{S.E.M.} = \left[\frac{\Sigma(X - \bar{x})^2}{n(n-1)} \right]^{1/2}$$

where:

X = the value of a replicate within a treatment

$\bar{x}$ = the mean of a treatment, and

n = the number of experimental subjects per treatment.

Numerical data were reported as the mean $\pm$ S.E.M. The difference between means was reported to be significant only if the calculated Student's *t* value was larger than that value of the

Student's *t* distribution where the probability (P) of a larger *t* value was less than 0.05.

II. Litchfield-Wilcoxon Method of Graphic Evaluation of Dose-Effect Relationships

The method described by Litchfield and Wilcoxon (1949) was utilized to estimate the median effective dose and 95% confidence limits for statistical evaluation of data from electroshock seizure threshold studies. This method provided a simple graphic analysis of raw data.

Percent response versus dose was plotted on logarithmic probability paper. The best fit line was estimated and drawn through data points. Tables and nomographs, provided with this method, were utilized to improve the position of the line and a $(Chi)^2$ test was employed to verify adequateness of fit of the line to the data points at a 95% confidence level. Median effective dose was read directly from the graph and the 95% confidence interval was calculated by using the nomographs provided.

The potency ratio (Median $dose_1$ divided by Median $dose_2$) between two median effective doses was analyzed by means of nomographs to determine if the two doses differed significantly from each other at a probability of 0.05. Numerical data were reported as median effective dose and 95% confidence interval.

For a more detailed description of this procedure, refer to the original paper by Litchfield and Wilcoxon (1949).

APPENDIX F

DRUG SOLUTIONS

Bicuculline: (K & K Labs, Inc.)

Route: Microinjection into inferior colliculi

Vehicle: Normal Saline

Dose: 0.55 mμmoles/IC

Concentration: 0.2 μg/0.5 μl (0.04%)

Dose: 2.45 mμmoles/IC

Concentration: 0.9 μg/0.5 μl (0.18%)

Dose: 1.91 mμmoles/IC

Concentration: 0.7 μg/0.5 μl (0.14%)

Dose: 1.36 mμmoles/IC

Concentration: 0.5 μg/0.5 μl (0.10%)

Dose: 0.41 mμmoles/IC

Concentration: 0.15 μg/0.5 μl (0.03%)

γ-Aminobutyric Acid: Sigma Chemical Co.

Route: Microinjection into Inferior Colliculi

Vehicle: Deionized Water

Dose: 2.50 μmoles/IC

Concentration: 258 μg/0.5 μl

Dose: 1.25 μmoles/IC

Concentration: 129 μg/0.5 μl

Dose: 0.625 μmoles/IC

Concentration: 64.5 µg/0.5 µl

Dose: 0.312 µmoles/IC

Concentration: 32.3 µg/0.5 µl

Dose: 0.15 µmoles/IC

Concentration: 16.15 µg/0.5 µl

Isoniazid: Sigma Chemical

Route: Intraperitoneal

Vehicle: Deionized Water

Dose: 200 mg/kg

Concentration: 200 mg/2 ml

Dose: 100 mg/kg

Concentration: 100 mg/2 ml

Dose: 50 mg/kg

Concentration: 50 mg/2 ml

RO 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9, 10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo (a) quniolizine): Hoffman-LaRoche, Inc.

Route: Intraperitoneal

Vehicle: Normal Saline

Dose: 5 mg/kg

Concentration: 5 mg/2 ml

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