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Title: Audio-Conditioned Convulsive Response: A Neurophysiological and Neuropharmacological Investigation of Neonate Mice

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Sound produces a high incidence of convulsive behavior in certain strains of mice but others are resistant. However, susceptibility to sound-induced convulsions in a resistant strain (CF#1) was found to be markedly influenced by prior auditory-conditioning, the condition-test interval (number of days between the initial and subsequent exposure to sound), and age. Previous auditory stimulation was found to be absolutely essential for the genesis of convulsions. Incidence in 12 or 45-day old mice was about 5% at any interval. However, 18 day old mice subjected to brief intense auditory stimulation (audio-conditioning) and tested at intervals of 1, 2, 3, 4, or 5 days resulted in a high incidence of convulsions. Clonic-tonic convulsions characterized the seizures at the 2 or 3 day interval; but at 5 days only clonus was seen. Susceptibility to sound-induced seizures persists for a prolonged period if the animals are exposed to the bell repeatedly at 2 day intervals, or if the animal had once experienced a convulsion.

Proper selection of age, conditioning, and the condition-test interval produces convulsions that are reproducible and of a predictable incidence and severity.

Mice that had been audio-conditioned were subjected to a battery of standardized, seizure-evoking procedures and the responses interpreted in terms of neurophysiological mechanism. In addition, the effect of maturation on the development of sound induced, chemoshock and electroshock convulsion threshold and pattern was studied. The data indicate that the pattern of maximal seizures induced by electroshock, pentylenetetrazol and sound are remarkably similar at a given age. It is suggested that the motor pattern in a seizure is limited at the spinal level and that maximal spinal activity can occur with a submaximal brain discharge. The threshold for low frequency electroshock and for minimal pentylenetetrazol decreased with increased age and weight. The low frequency electroshock and minimal pentylenetetrazol threshold are measurements of similar neuronal mechanism. There was no significant difference in maximal electroshock seizure threshold with aging but duration of the seizure pattern decreased with increased age. Mice that had been audio-conditioned responded to the test stimulus with a maximal seizure provided they were between 18 and 23 days of age. It may not be a matter of losing sensitivity to the initial sound but rather a matter of the development of a more prominent

system such as an inhibitory system. An inherent difference in the adaptation of an audiogenic seizures susceptible strain (DBA/2J) and CF#1 mice was demonstrated by the fact that the DBA/2J mice did not become seizure resistant after chronic exposure to intense sound. Audio-conditioning produced profound changes in the ontogenic development of low frequency and chemoshock seizure threshold, but maximal seizure threshold and pattern were unaffected. Drugs which effectively modify maximal audiogenic seizure pattern, the anticonvulsant and tranquilizers, are also effective in suppresssing audio-conditioned convulsions. No drug negated the effects of audio-conditioning. Anoxia was unique among nonauditory stimuli in that it elicited audio-sensitivity. The audio-sensitized CF#1 mice differ in at least two ways from littermates. First, the oscillator mechanism of the audio-sensitive mice is more easily discharged by electric and chemical stimulation, and second, there is an alteration in the neuro-ontogenic pattern. It was concluded that the Audio-conditioned Convulsive Response has several potential applications, and is a simple but reliable test in assessing the degree of neural maturation of the mouse.

AUDIO-CONDITIONED CONVULSIVE RESPONSE: A NEUROPHYSIOLOGICAL AND NEUROPHARMACOLOGICAL INVESTIGATION OF NEONATE MICE

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GENERAL INTRODUCTION

Although the production of anatomical defects by drugs administered during gestation has attracted international attention, much of it stemming from the German sleeping pill tragedy, the pregnant woman remains an uncommonly chosen candidate for drug administration. A 1963 federal report (Peckham, 1963) states that 92% of the women surveyed received at least one prescription drug during pregnancy and that 3.9% were given 10 or more drugs by their physician. This is an alarming rate in view of the fact that it no doubt represents a dramatic reduction in the rate administered prior to the 1961 world wide panic precipated by thalidomide. Drug administration during pregnancy is apparently increasing rather than declining for it was recently reported (Nora et al., 1967) that a mean of 3.1 drugs were prescribed to women during their vulnerable first trimester and 5.4 drugs were given during pregnancy. The irony of the situation is the possibility that much of the less obvious teratogenic influences, not only of drugs but also that by environmental pollution, may be passing generally unrecognized. In fact the potential of other than anatomical defects has only begun to be investigated. Some investigators (Werboff and Gottlieb,

1963; Hamilton, 1945) would like to implicate another system susceptible to teratogenic effects of drugs. That is, the behavioral aspects, or functional adaptation of the offspring to its environment, to distinguish it from the gross effect of mental retardation. Indeed there is mounting evidence that drugs administered during pregnancy may effect postnatal physiological and behavioral development without inducing morphological defects (Murai, 1966; Ordy et al., 1963, 1966; Kietzkin, 1964; Ader and Belfer, 1962; Campbell, 1965; Thompson and Quinby, 1964; Hoffeld and Webster, 1965; Al-Hachim and Fink, 1967; Jewett and Norton, 1966; Young, 1964, 1964b; and the extensive investigations from Werboff and co-workers 1961, 1961b, 1962, 1962b, 1963; Havlena and Werboff, 1963). However much of the work in these reports are generally inconclusive and rather disappointing in the interpretive value of the results obtained. The major difficulty appears to be a lack of suitable standardized procedures or at least a single common basis to aid in the evaluation of the conclusions evolved. Since the literature search was completed a review of the psychological investigations (Young, 1967) has been published and similar criticism expressed.

This investigation is an attempt to develop a rapid and reliable model for study and assessment of the potential of perinatal drug therapy to elicit postnatal behavioral alteration. Inasmuch as postnatal neurogenesis is a rapid

process involving several time and space dependent interlocking systems, it was necessary to establish certain criteria for "normality" before any indication of an altered pattern of nervous system maturation could be established.

An oversimplified illustration (Figure 1) is presented as an overview of the area of investigational concern and indicates the multitude of complex interacting processeses which are involved in prenatal and postnatal development. These events are all carefully controlled in relation to the time and space denoted by the cogwheels. Any displacement, either in time or space may result in malformation. Mendelian Laws suggest that any genetic mutation will lead to a predictable frequency of abnormalities in the offspring (Frazer, 1964). Not nearly as predictable, however, would be interference with the almost countless mechanisms involved in the long, complex chain of events of organogenesis and postnatal development of neurological and behavioral systems. However all of the events involved are within the influential sphere of hereditary control. That is to say that normal environmental factors or teratogenic agents cannot work outside the tempering influence of the hereditary machinery of the organism (Smithberg, 1967). How this interdependence is accomplished has been and undoubtly will remain in the forefront of biological research. Investigators from virtually every discipline of the biological sciences are

Figure Legend for Figure 1.

Diagram illustrating the Complex Chain of Processess related to the Development of Normal or Abnormal Structure or Behavior.

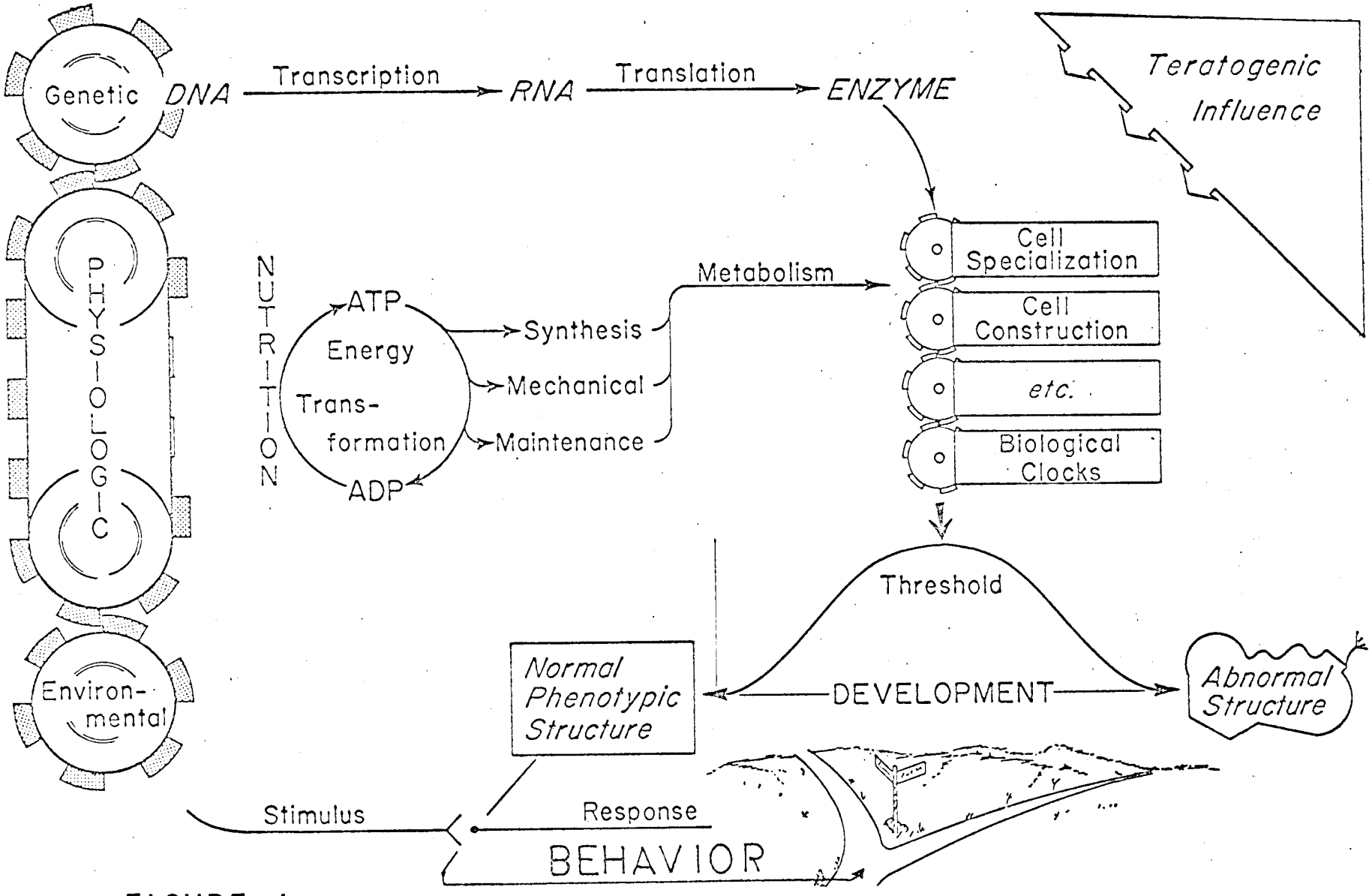
Genetic
DNA
Transcription
RNA
Translation
ENZYME
Teratogenic Influence
PHYSIOLOGIC
NUTRITION
ATP
Energy
Trans-
formation
ADP
Synthesis
Mechanical
Maintenance
Metabolism
Cell Specialization
Cell Construction
etc.
Biological Clocks
Threshold
Environ-
mental
Normal Phenotypic Structure
DEVELOPMENT
Abnormal Structure
Stimulus
Response
BEHAVIOR

FIGURE 1.

engaged in some aspect of this problem. Indeed a large number of pharmacologists are quite active in various aspects.

A number of recent reviews indicate the dramatic "change in thought" in the science of experimental teratology since 1961. Reviews on the physiologic (Done, 1966; Nyhan and Lampert, 1965; Yaffee and Back, 1966; Sutherland and Light, 1965; Friend, 1963; Cohlan, 1964; Meester, 1964; Apgar, 1964; Connelly, 1964; Werboff and Gottlieb, 1963; and Willner, 1965) and the teratologic (Karnofsky, 1965; Kalter, 1965; Peck, 1963; Kalter and Warkany, 1958; Smithells, 1966; Smith, 1966; Sallomi, 1966; and Cahen, 1964) influences of drugs upon the fetus and infant reflect such a revolution. The term teratology is now being used in the sense to denote the entire subject of structural or functional departures from the normal, for as Browne (1967) succinctly denoted: that an inborn metabolic defect is just as much teratological as a missing head.

The general areas of investigational concern of the pharmacologist are rather apparent in that immature mammals (the neonate or perinate) are more sensitive to infections, poisons, drugs and diverse environmental factors than are mature mammals. Infants may have alarming toxic reactions to drugs whose effect on adults are very mild, even when appropriate dosage corrections for body size, weight, and surface area are made (Nyhan, 1961; and Shirkey, 1965).

The differences between adults and infants are so profound and occur so frequently that the physiological and the pharmacological responses of the fetus and the infant are sufficiently novel to those of the adult to warrant a separate classification. Many of the syndromes resulting from therapy in this age group go unrecognized and further treatment will probably result in new types of drug toxicity (Montagu, 1962; and Nyhan and Lampert, 1965). The need for the prevention of such problems indicates the importance of a systematic experimental investigation of the pharmacologic differences between adults and infants.

In order to provide a proper perspective it must be stated that "drugs in general" are not an important (or at least recognized) factor in congenital malformation (Warkany, 1966; Green, 1964; Nora et al., 1967). Nevertheless the etiology of approximately 90% of the human coginital malformations is unknown. Additional research on radiation, viral infections, and non-drug chemicals may be expected to establish several causal relationships (Nyhan and Lampert, 1965). The primary reason for suggesting drug use be limited during pregnancy is that at the present time there is not sufficient information available to make a valid judgment on the question of human malformation. This is especially true in view of the magnitude of exposures (Nora et al., 1967) and the potential for drug interaction. Perinatal and infant mortality could also be used to

evaluate the scope of the potential hazard. A recent report, (Chase, 1967) indicates that infant mortality in the United States is no longer declining, while in other countries a more favorable level of mortality continues. Chief among the direct causes of perinatal and infant death are respiratory malfunction, premature birth, and congenital malformation of the heart, digestive and central nervous systems. Such reports implicate the need for further investigation to elucidate the cause-and-effect relationship of infant mortality; not to mention nonfatal malformation.

Before pursuing the assessment of the suggestion of "behavioral teratology", it may enhance clarity to dispel some discredited but wide spread beliefs. The first is the belief that the fetus, in its protected amniotic environment is immune from insult. Although a placental barrier in the fetal-maternal relationship exists its function is limited. In thinking about drugs which affect the fetus, our concern is whether the agent crosses the barrier and if it will produce an effect. Much of our thinking regarding the placenta has been dominated by the concept that it is a physical barrier to diffusion--that the number of layers of cells is inversely related to the rate a substance traverses the placenta (Villee, 1967). If the placenta were simply a semipermeable membrane and diffusion the only force involved in transfer of substances from mother to fetus, this would follow. However neither of

these is true. Placentas are composed of several types of cells, and have thick and thin spots (Mossman, 1953). More importantly, the transfer of molecules, other than oxygen and carbon dioxide, occur largely by forces other than simple physical diffusion (Villee, 1967). In addition it should be remembered that a drug may harm the fetus indirectly by effecting the placenta (West, 1962), and thus interupt the essential role it plays in synthesis and storage of molecules important to both the fetus and the maternal organism. The functional capacity of the placenta in transporting material is regulated not by its thickness but by the metabolic activity of the cells present (Villee, 1963). Not only do the placentas of different species differ in cell types and numbers but an individual placenta differs in thickness and metabolic potential at different stages in gestation as certain types of cells appear, increase, or disappear (Mossman, 1953; and Villee, 1963, and 1967). The metabolic potential and overall function of the organ undergoes extensive changes in the course of development. That is to say, as the fetus grows the functional capacity of the placenta appears to increase to keep pace with his changing needs.

A voluminous literature (Moya and Thorndike, 1962; Moya and Smith, 1965; Villee, 1964; Dentkos, 1966; Klevit, 1966) clearly demonstrates that all types of molecules, except very large ones, can in one way or another cross

the placenta. An important difference between potentially hazardous and safe drugs is the rate of transfer. With certain drugs this rate is so low that the amount transferred becomes pharmacologically undectable. The fetus itself has only limited power for accomodation of any type. However the effect upon the fetus depends in part on the stage of development at the time of drug exposure (Wilson, 1965). The fetal effects of a drug depends not only on its rate of transfer but also on its rate of degradation and excretion as well as the sensitivity of the fetus in a particular stage of development. Even though the placenta is not the all guarding sentinel as once thought, it is a remarkable structure discharging its responsibility so effectively that 95-98% of infants enjoy precisely optimum conditions (Apgar, 1964; Nyhan and Lampert, 1965). It is indeed unfortunate that drugs are given so indiscretely that they might cause malformation.

The second belief to consider is that the fetus and the newborn have the same facility to metabolize and detoxify substances as the adult. Their inability to metabolize a wide variety of drugs such as hexobarbital, phenacetin, aminopyrine, antibiotics and many others has been extensively documented since Jondorf, Maickel and Brodie's (1959) original report (Hart et al., 1962; Stave, 1964, 1965; Vest, 1965; Fouts et al., 1959). For example 5 day old mice sleep 50 times longer than adults given

pentobarbital even after dosage adjustments for all factors except age (Jondorf et al., 1959; Catz and Yaffe, 1967). The absence of sufficient hepatic metabolism may be the primary factor contributing to such abnormal responses. The hepatic microsomal enzymes, which are responsible for termination of action of a wide variety of drugs, are almost totally lacking in the newborn (Hart et al., 1962; Catz and Yaffe, 1967). The development time to adult levels varies with the different species and with the particular enzyme. Metabolic differences between the immature and the adult are both quantitative and qualitative in nature (Lee, 1966). Furthermore the inherent response of the organism may differ with age (Key and Marley, 1962). The differences in metabolism is further complicated by differences in distribution, excretion, and absorption due to, among other factors, immaturity of the kidney, high fat content and lower body temperature. Membrane permeability also changes with prenatal and postnatal age. For example kernicterus, an anomaly in the newborn whereby a sulfa drug displaces protein bound bilirubin, the free bilirubin penetrates the immature blood brain barrier and results in brain damage (Odell, 1959; Menken, et al. 1966). The Blood Brain Barrier is not complete in the neonate but appears to have limited function which increases with postnatal aging (Himwich, 1962; Frohlich and Mirsky, 1942). Phenobarbital has been observed to

penetrate the brain of a kitten much more rapidly than that of a cat (Domek et al., 1960); there appears to be an inverse relationship between the degree of myelination and the cerebral drug concentration attained. An increasing resistance of the animal to barbiturate hypnosis during the first three weeks of life is accompanied by a decreasing ability to withstand anoxia (Done, 1966; Montagu, 1962). The brain progresses from a relative dependence on glycolysis as an energy source to a predominately oxidative metabolism. Thus idioacetate (an inhibitor of glycolysis) is relatively effective in inhibiting neonatal brain metabolism while malonate, which inhibits oxidative pathways through inhibition of succinic dehydrogenase, becomes more effective with maturity. This is not to imply that anoxia (hypoxia) is innocuous to the fetus or newborn. In fact fetal anoxia, whether drug or otherwise induced, results in damage in many organs and may range from severe to slight such as extensive brain damage to a slight reduction in mental ability (Montagu, 1962.)

A final word on teratology, both pre- and postnatally expressed forms, must mention studies in laboratory animals are of uncertain relevance to man. However, careful selection of the animal--that is fitting the animal to the experiment--does offer useful information to mechanisms by which abnormality is produced (Warkany, 1965). In terms of the present project the extensive time factor and

complex postnatal environment of humans would make an extremely difficult task to relate a behavioral deficit to prenatal drug administration.

The relatively few studies concerned with the behavioral consequences of prenatal or postnatal drug administration are somewhat crude, and at best merely suggestive. Most of the research has been conducted from the "I wonder what would happen" approach (Young, 1967). There are also other methodological problems, such as nearly universal use of a single dosage level rather arbitarly obtained. Over 90% of the studies reviewed used reserpine, chlorpromazine, meprobamate or all three drugs. These drugs have the common property of affecting several different systems and have essentially unknown primary site or mode of action.

An even more serious methodological problem, in view that most reports originated from psychological investigators, is that a common behavioral method utilized, the open field, ("index of emotionality") lacks relability and validity (Young, 1967). Most of the studies only used a single behavioral test, and did not attempt to determine any chemical or physiological variables. Two exceptions are the study of Murai, (1966) and the intradisciplinary investigation of Ordy et al., (1967). Still another issue not generally considered is the ontogenic differences of the behavioral task. Generally a single arbitrary age was

selected. To accurately assess a behavioral deficit an alteration in the time-space relationship should be established; much as the teratologist have established in anatomical dysmorphology.

Birth of mammals is an abrupt and definite event, however the function of most organs are relatively immature at birth; the exact degree of immaturity varies from specie to specie. It is therefore not illogical that the time-space relationships emphasized during embryonic development should also be relatively important during postnatal development. Indeed the concept of a "critical period", originally used in embryology, has been applied to postnatal maturation of animal behavior (Fox, 1966).

The neuro-ontogenic critical period has been defined as that time the neurons approach maturity as judged by certain anatomical changes (Flexner, 1955), socialization (Scott and Marston, 1950), reflex-ontogeny (Fox, 1965), and neurological and behavioral tests (Himwich, 1962). Several recent reviews have been published on neuro-behavioral development (Himwich, 1962; Scott, 1962, Hamburger, 1963; Denenberg, 1964; Fox, 1966; Levine and Mullins, 1966; Barnes, 1967; Fuller and Wimer, 1966; and Fuller, 1967). Thus a great deal of knowledge has accumulated about the developing nervous system and the correlation of reflex and behavioral changes. Much of this knowledge stems from

studies on infantile handling which is found to reduce "emotionality", to change adrenal response to stress and to accelerate maturation. However, there are many conflicting and inconsistent findings in this field; this stems largely from the number of variables and theories involved (Richards, 1966).

The application of behavioral critical period tests (Fuller, 1967; Scott, 1962) and reflex-ontogenic tests, (Fox 1966) have been used as indicators of normal development in mice (Fox, 1965; Scudder et al., 1967), humans (Apgar, 1966; Drage and Berendes, 1966), cats, dogs, and other laboratory animals (reviewed by Fox, 1966). However few definitive studies have been reported that utilize an alteration in the time relation of neurophysiological development as an indication of experimental treatment effect (e.g. giving animals drugs, radiation or malnutrition during early development). A survey of the literature suggests that the animal studies that have dealt with the experimental treatment in early life upon adult behavior are disappointing, both in number and in the interpretive value of the results that have been obtained. Furthermore the survey indicates a need for simple, but reliable tests that could serve as an indicator of normal neuro-behavioral development.

The reflex-ontogeny tests apparently would offer the most interpretive information, however, they are tedious

and extremely time consuming. Sound induced convulsions, or audiogenic seizures, are known to be modified by age and are believed by some investigators (Chance, 1963) to be a type of reflex epilepsy. Castellion (1964) used maturation of seizure susceptibility to study the audiogenic seizure. Thus, for the present investigation, it was surmised that sound induced seizures might be used as a reflex-ontogenic test.

Numerous investigators have unsuccessfully attempted to define the mechanism underlying the audiogenic seizure and there are over 600 such reports in the literature. There has not been extensive review of convulsions induced by sound since 1955 (Bevan); however, the 1963 international symposium on the audiogenic crisis (Colloq. Int. Cent. Nat. Recherche Sci. volume 112); the symposium on Bio-Acoustics (Lehmamn and Busnel, 1963); and the digest of Fuller and Wimer (1966) minimize the need for such a review. In brief, the susceptibility to audiogenic seizure is controlled by hereditary traits and only certain strains of rodents are susceptible.

It has been observed that a cross between a field mouse and a laboratory mouse (CF#1) produces offspring that were susceptible to sound induced seizures (Iturrian, 1962 unpublished).

A surprising discovery (Fink, 1964 personal communication) was that young CF#1 mice are susceptible to

audiogenic seizures; this stock was generally felt to be non-susceptible. Indeed the CF#1 stock had been used as control mice for studies of the audiogenic seizure (Fink and Swinyard, 1959; Swinyard et al., 1963; Castellion et al., 1965). Al-Hachim (1965) used audiogenic seizures to interpret behavioral effects of a pesticide given during gestation. During a similar investigation (Iturrian, 1965 unpublished) the age of the mice to be tested with sound was changed to avoid stunting the mice. No convulsions resulted. It was then recognized that few convulsions occurred during the first exposure of CF#1 mice to sound and that subsequent exposure was necessary to induce seizure activity.

In view of these observations it was thought that this convulsive phenomenon might be a useful indicator of a critical period during neuro-behavioral development. This investigation is an attempt to establish certain criteria for "normality" of convulsive responses during postnatal development, and the interpretation of these responses in terms of underlying neurophysiological and neuropharmacological mechanisms. Such mechanisms may provide a fresh perspective to postnatal behavioral development and an assessment of perinatal drug therapy as a potential for behavioral teratogenicity. It was anticipated that the phenomenon might also be of some value in interpreting the mechanism of sound induced convulsions.

GENERAL PROCEDURE

All experiments employed mice that were bred and raised in our laboratory. The mice used were first generation from parents purchased from Carworth Farms. Since it was deemed important to control experimental variability, large numbers of mice of a given age were required. This was accomplished by modification of the Whitten effect (1959) as suggested by Ross (1961). The males were housed individually prior to mating to minimize fighting and to insure healthy bucks. No difference in fertility was noted if breeding occured in the bucks home cage or the colony cage, therefore the males were introduced into the colony cage to economize on labor and time. The female mice were housed 5 per cage and a proven breeding male was added to the cage at 4 p. m. The male was removed four days later. Over 70% of the mice were found to breed during the 3rd or 4th night, which is in agreement with results published by Whitten (1959) and Ross (1961). When it was desirable to have mating occur during a single night, the females were exposed to a caged male (no contact allowed) for two days prior to pairing with the male.

Three days prior to expected parturation two or three pregnant females were housed per cage to minimize the individual maternal differences (Evans, 1962). All cages were provided with a small amount of newspaper (approximately 1 gm) to serve as nesting material. The cages were

examined for offspring every 6 hours. The animals were considered newly born when found, and the litters were grouped so that 6 hours was the maximum variability in age within an individual cage.

The mice were raised under conditions of controlled lighting, humidity and temperature in quiet animal quarters, and were housed in plastic disposable cages with wire tops (Maryland Plastics cage #22). The animal quarters were kept on a cycle of 12 hours of light followed by 12 hours of darkness. The light cycle started at 10 a.m. to allow ample time for experimental procedures. A one and one-half inch strip of masking tape was placed around the top of the cage to reinforce it and make it darker. This procedure greatly prolonged the utility of the cage. The mice were littered with a processed sawdust bedding as other bedding material was less satisfactory since reproductive capacity was reduced (Iturrian and Fink, 1968). Growth rates were determined on day 4, 9, 12, 15, and 18 after birth. All mice which showed symptoms of disease as well as any animals which were unthriftly or grossly undersized were classified as culls and not used in the experiments. The mice were not weaned until they attained 30 days of age. They had free access to Purina Laboratory chow and water. A teaspoonful of cracked wheat was added to each cage once daily to calm the mother and for nutritional reasons.

Since one of the parameters of the experimental design was sound the animals were carefully protected from environmental noise.

Mice of the desired age were placed in a glass chamber (25 cm diameter and 15 cm deep) and subjected to 60 seconds of sound (95 db relative to 2 x 10^{-4} dyne/cm^2) produced by an electric door bell (2 1/2 inch diameter) which was activated by a 3.6 volt transformer. Upon the first exposure to sound (audio-conditioning) the mice displayed an auricular startle as described by Fox (1965). Those few subjects convulsing upon the first exposure to sound were discarded. All experimental procedures involving sound were conducted between the 8th and 12th hour of the daily 12-hour light cycle, in view of the circadian nature of the audiogenic seizure.

If the initial exposure to the bell affected grouped animals in the same manner as individual mice, experiments based on this phenomenon could be considerably facilitated time-wise. In order to determine this point, 3 groups of 8-10 mice each were placed in the conditioning-chamber at one time and the entire group subjected to 60 seconds of sound. No differences were detected between mice that were audio-conditioned individally or in groups. However, upon subsequent exposure to sound (test) seizure incidence was markedly enhanced if the test bell was presented to

aggregated mice. Therefore all mice were audio-conditioned in groups and tested individually.

The mice were exposed to sound a second time (test) after the lapse of 1 to 5 days (conditioning-test interval). Since even brief restraint and handling may affect seizure threshold or severity, care was exercised not to arouse the young mice unduly as they were individually removed from the nest cage and placed in the test chamber. After an observation period of 30 seconds the bell (test stimulus) was turned on for 60 seconds or until the subject convulsed. After an initial startle response to the onset of the sound, any number of behavioral patterns may appear. Some mice exhibit a restless type of movement, typically consisting of jerky sidling or backward steps; other mice crouch in a motionless cringe, a few appear to ignore the sound. It is worth noting that mice which exhibit a motionless crouch usually were those which later had a maximal seizure, while those which exhibit restless movements were animals that developed minimal seizures.

Seizure activity begins after a latency of a few seconds with an explosive burst of wild running which continues for 3-5 seconds. The running episode abruptly ceases when the mouse appears to leap into the air (and occasionally emits an audible cry) which is succeeded by a convulsive spasm, a clonic convulsion, or a clonic-tonic convulsion. In trials culminating in a clonic-tonic seizure, the

mouse runs, loses consciousness, falls on its side and the feet may kick incoordinately. This phase continues for 2-3 seconds, and the mouse draws up the hind legs (tonic-flexion) and then thrusts them out and back vigorously (tonic-extension). At the same time the front legs are drawn together tonically over the chest, the tail is extended stiffly behind, the eyes are closed, and the ears are folded tightly against the head. This position is held for about 15 seconds, then the abdominal muscles relax, the ears return to the normal erect position, the tail twitches and the animal is dead. However, recovery from the seizure occurs if the animal starts to breathe when the abdominal muscles relax. A period of terminal clonus follows which ends in a cataleptic state. This cataleptic state is stable in regards to most external stimuli and disappears under the influence of auditory stimuli. Behavior in the post-convulsive phase consists of a variety of motor phenomena including a syndrome that may be described as epileptoid furor.

The severity of the seizure may be illustrated by a severity scale shown in Table 1 (suggested by Wilson, 1963).

Table 1. Severity of seizure response.

Response	Severity
preconvulsive	0
wild running	threshold
convulsive spasm	↓
clonic convulsion	minimal
tonic-flexion	↓
tonic-extension	maximal
relaxation or death	
post convulsive	

A maximal seizure response is characterized by hind leg tonic extension; a minimal seizure is characterized by only clonic activity. Total incidence includes threshold convulsive behavior as well as minimal or maximal responses. The animal may die after the maximal response.

Estimates of severity of experimental convulsions have also been derived by: latency measures (Frings et al., 1953; Fuller and Smith, 1953); duration of running (Bevan and Hunt, 1953), flexion (Woodbury et al., 1952) and extension (Tedeschi et al., 1956). Indices of severity derived from latency and duration measurement is shown in Table 2.

Table 2. Indices of seizure severity by measurement of latency and seizure duration.

Measurement	Index of severity
Latency	
to running (decreased)	↑(increased severity)
to tonic-extension (decreased)	↑(increased severity)
Duration	
running (decreased)	↑(increased severity
initial clonus (decreased)[a]	↑(increased severity)
tonic-extension (decreased)	↑(decreased severity)

a) includes duration of tonic flexion.

No one has attempted to devise a chronaxie-like score that incorporates both seizure intensity and latency.

The type of seizure response was recorded; the latency of wild running (time from the start of bell to onset of running), latency to end of the running episode, and latency to tonic-extension (from the start of the bell to onset of hind leg tonic extension) was measured to the nearest 1/10 second with an electric timer for each mouse. The end points employed are those suggested for mice by Fink and Swinyard (1959). No attempt was made to resuscitate animals if respiratory difficulty occurred. The test stimulus was terminated as soon as the seizure progressed beyond the wild running phase. The duration of the various components was obtained by subtraction from seizure latencies.

Sound induced seizures have not been standardized because of the many physiological and psychological factors which alter seizure incidence and severity (Bevan, 1955). In fact seizure susceptibility is effected by such a variety of factors that it is difficult to duplicate experiments quantitatively (Fuller and Wimer, 1966). The primary difficulty may be that the stimulus lacks sufficiently precise definition; little concern has been directed toward further specifying the physical properties of the convulsion-provoking sound or to clarify the significance of nonauditory characteristics (Bevan, 1955). Fuller and Wimer (1966) suggest that the physical dimensions of the stimulus are not critical determinants provided it is sufficiently intense and of proper frequency range.

However we have found (Iturrian and Fink, 1968b) that the intensity of the sound producing the audiosensitivity influences the incidence and severity of sound induced seizures. Therefore, mmere measurement of the intensity of the sound level may not be sufficient standardization from bell to bell as other characteristics are apparently important determinants of seizure pattern. Furthermore, the bell apparently changes tone after extensive use as shown by a decreased incidence of maximal seizures (Table 3). In addition some new bells were rather ineffective. The latency of the different seizure components was also affected as the bell changed tone (Table 4). An increased latency reflects

Table 3. Indices of seizure severity by measurement of latency and seizure duration.

Date	Source of the initial sound (Bell #)	Approximate no. hours	CONVULSIVE Response (%) Minimal	Maximal	Total	No. Animals (18 days of age) Exposed
			----------response-----			
11/18	#5	30	14	86	100	14
3/27	#7	30	14	71	86	7
4/12	#7	50	7	75	89	28
6/26	#8	70	6	62	75	16
6/27	#8 (test #9)	0	30	70	100	10
7/12	#9	30	8	92	100	12
7/13	#9	40	17	75	83	12
7/14	#9	50	30	50	90	10
7/20	#9[a]	120	63	25	87	8
7/12	#9 (test #10)	0	17	67	83	6
7/16	#10	15	14	71	86	7
8/13	#10 (test #11)[a]	0	30	30	90	10

a) Bell discarded as not minimally effective.

Table 4. Duration of bell use and effect on latency.

Source of the initial sound Bell#	Hours rung	LATENCY[a] TO SEIZURE COMPONENT				DURATION OF Initial-Clonus[b]
		Running	Minimal	Initial Clonus	Extension	
#7	50	6.3 ±.4	10.7 ±.9	12.9 ±1.9	15.7 ±1.8	2.6 ±.3
#8	70	6.5 ±.3	--------	11.1 ±.2	15.2 ±.6	4.0 ±.2
#8 (test #9)	0	11.6±1.3	20.6 ±2.1	13.9 ±.6	17.4 ±1.9	3.5 ±.3
#9	30	7.1 ±.4	28.1 ±7.1	10.3 ±.4	13.0 ±.4	2.8 ±.2
#9	40	8.5 ±.6	---------	12.3 ±.5	16.2 ±1.8	3.8 ±.8
#9	50	7.8 ±.6	---------	12.0 ±1.5	15.4 ±.8	3.1 ±.9
#10	15	8.2 ±.3	---------	12.3 ±.6	14.9 ±1.6	2.9 ±.7
#10 (test # 11)[c]	0	4.3 ±.4	27.0 ±12.4	7.6 ±.3	10.9 ±.5	3.3 ±.2

a) Time in seconds (± standard error) from the start of the bell to onset of response component.
b) Includes duration of tonic-flexion.
c) Bell discarded as not minimally effective.

decreasing seizure severity and/or susceptibility. The duration of initial clonus is an additional measure of severity and also demonstrated decreased severity after extensive use of a bell.

Despite the variety of factors that affect seizure susceptibility and/or severity it is possible to standardize the procedure for sound induced convulsions if proper precautions are enacted. Therefore, each bell was checked biologically to ascertain its effectiveness. The criteria was about 90 percent seizure incidence with 60 percent maximal seizures among 20 day old CF#1 mice tested 48 hours after the initial exposure to the bell.

Total incidence and latency of seizures between bells and even different groups of mice appear to be remarkably constant (Table 3 and 4). No differences in seizure susceptibility between sexes were noted and the season of the year apparently does not effect the incidence of convulsive response. The variation observed in seizure severity and latency are attributed primarily to changes that occur in the source of sound eliciting the audio-sensitivity and subsequent convulsions. Bells were periodically checked biologically to provide a reference of responses throughout the duration of the investigation. A bell was discarded when it failed to be effective as measured by the criteria described. Since severity of the response is affected by both the audio-conditioning and the test stimulus, a

replacement bell was obtained and tested prior to a bell's loss of apparent effectiveness.

EFFECT OF AGE AND CONDITIONING-TEST INTERVAL (DAYS) ON AN AUDIO-CONDITIONED CONVULSIVE RESPONSE

INTRODUCTION

The research potential of sound-induced seizures for investigating drug action or maturation of the central nervous system has limitations due to the complexities involved in maintaining susceptible animals. Animals are considered susceptible if they exhibit a clonic-tonic seizure when exposed to sound only once (Vicari, 1951; Fuller et al., 1950). Nearly all (90 - 100%) inbred mice of genetically-susceptible strains, for example the DBA/2 strain, exhibit seizures when first subjected to sound, provided they are between 15 and 35 days of age (Frings et al., 1951; Vicari, 1950) and death usually follows the occurrence of a maximal seizure (Hall, 1947; Vicari, 1947; Lieblich and Guttman, 1965).

Pretest conditioning by sound enhances or reduces susceptibility depending upon the temporal parameters of the treatment (Ginsburg, 1963; Fuller and Wimer, 1966). "The classical priming method of physiology has shown the number of animals convulsing varies with the duration of the conditioning stimulus and the condition-test stimulation interval" according to Bevan (1955). Generally both the duration of the conditioning stimulus and conditioning-test interval have been short, being only a few seconds

(Fuller and Smith, 1953; Bevan, 1955). However, spacing tests at a predetermined interval of days increases seizure incidence among mice of certain strains (Iturrian and Fink, 1967, and unpublished; Henry 1967).

This investigation is concerned with production of a high incidence of convulsions without using genetically susceptible strains, special diets, chemicals, or surgical manipulation. One objective was the assessment of the research potential of these convulsions for experiments in physiology and pharmacology. Therefore it was important to search for a predictable incidence of maximal seizures and a low death risk. The mice used were a strain (CF#1) that has a low occurrence of death following electroconvulsive shock (Torchiana and Stone, 1959; Swinyard et al., 1963).

METHODS

Ninety-eight different groups, a total of 1587 CF#1 mice, of the desired age were obtained by the method described in the General Procedure. The groups were audio-conditioned (initial exposure to sound) and exposed to sound (test) again after a lapse of 1 to 5 days (conditioning-test interval). The method of testing and audio-conditioning is described under General Procedures. Since the primary objective was to obtain a predictable incidence of maximal seizures the groups were preferentially assigned

to the age and conditioning-test interval that resulted in higher incidence of maximal responses.

Seizure activity began with intense running, and was usually succeeded by a convulsive spasm, a clonic convulsion, or a clonic-tonic convulsion. The tonic convulsion is characterized by hindleg extension, and the animal may die after such a tonic, or maximal seizure. The minimal response is characterized by only clonic activity. Total incidence includes threshold convulsive behavior as well as minimal or maximal responses. The incidence of the convulsive response is expressed as a percentage of the total number of mice tested. The type of seizure, the latency of wild running (time from start of bell to onset of running), duration of running and the latency to tonic extension (from the start of bell to onset of hindleg tonus) was recorded for each mouse.

RESULTS

Sound resistant strains, such as the CF#1, show a convulsive incidence of less than 5 percent upon the first exposure to sound; this incidence is independent of age. However, upon the second exposure to sound 3 days after the initial exposure, the incidence of convulsive behavior was greatly altered and was found to be profoundly influenced by age (Table 5). Previous auditory stimulation was found to be absolutely essential for the genesis of convulsions.

Table 5. Effect of Age and Prior Auditory Stimulation upon Convulsions.

AGE (days) when Conditioned	CONVULSIONS (%) First exposure to sound	CONVULSIONS (%) Second exposure 3 days later	Number of Animals
12	0	0	53
20	4	90 ± 4[a] (tonus 65 ± 7)	63
30	8	31 ± 3 (tonus 3)	61
45	5	3	92
60	2	2	97

(a. ± standard error).

Twenty day old mice audio-conditioned 3 days previously exhibited 90 percent seizure activity; a high percent of the animals tested had maximal seizures. At 30 days of age total incidence had decreased greatly and the seizures were predominately clonic. Results obtained on 60 day old mice were similar to the incidence obtained by testing groups of mice received from Carworth Farms.

To ascertain the relative reproducibility of the seizures, additional animals of the selected age were randomly assigned to various groups. They were audio-conditioned and exposed to the test stimulus 2 or 4 days later. The observed frequency of the various seizure components are recorded in Table 6. The incidence, pattern of seizure and uniformity of response between groups of a particular age and condition-test interval appear to be remarkably constant.

The audio-conditioned convulsive response was characterized in detail at the period of greatest susceptibility to maximal seizures (Figure 2). A total of 1182 animals of the desired age and weight were divided into groups of at least ten mice each and subjected to the initial 60 seconds of sound stimulus (audio-conditioning). The mice were then exposed to sound the second time at an interval of 1, 2, 3, 4, or 5 days later (conditioning-test interval). Mice audio-conditioned at 12 days of age or less did not exhibit seizures upon the second exposure to sound at any

Table 6. Reproducibility of Audio-Conditioned Convulsive Response. Each group is an independent sample.

AGE when Conditioned	18 days		18 days		22 days	
Condition-test	2 days		4 days		2 days	
Group	A	B	C	D	E	F
	CONVULSIVE RESPONSE (%)					
Total Incidence	86	91	45	42	48	46
Clonus	26	29	8	3	13	18
Maximal Seizure	60	55	37	33	22	24
Death	20	15	16	19	9	9
Number tested	50	60	36	38	23	33

Figure Legend for Figure 2.

Profile for Seizure Susceptibility among CF#1 mice on the Second Exposure to Sound. Seizure incidence on the first exposure to sound was less than 5% and those subjects were not tested further.

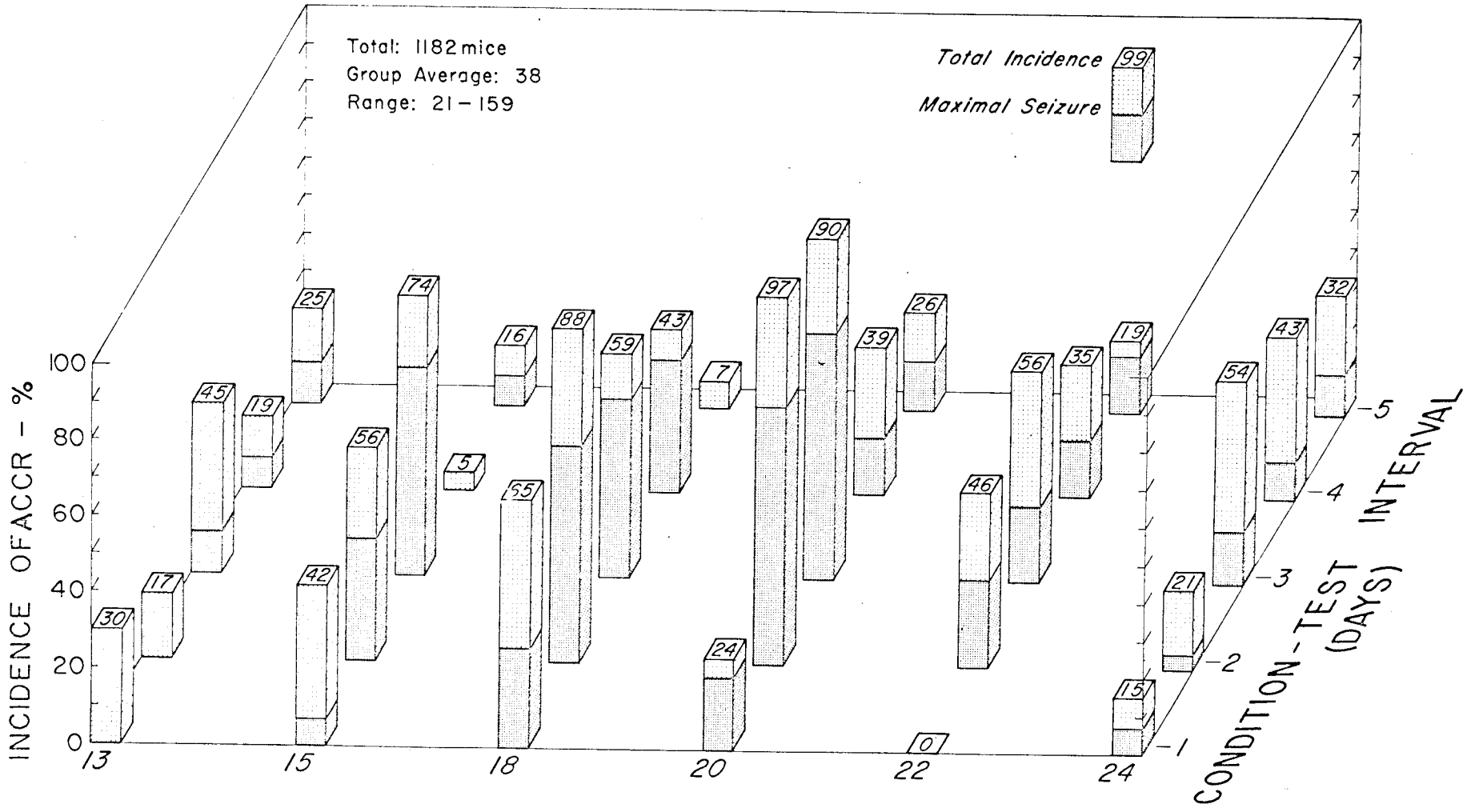
Total: 1182 mice
Group Average: 38
Range: 21 – 159
Total Incidence
Maximal Seizure
INCIDENCE OF ACCR - %
0
20
40
60
80
100
13
15
18
20
22
24
AGE WHEN FIRST EXPOSED TO SOUND - DAYS
CONDITION-TEST INTERVAL (DAYS)
1
2
3
4
5
30
17
45
19
25
42
56
74
5
55
16
88
59
43
24
7
97
90
39
26
46
56
35
19
15
21
54
43
32

FIGURE 2

conditioning-test interval. Total incidence of wild running not succeeded by a more severe convulsion was 5.8± .5% (S.E.).

Seizure was markedly influenced by the age of the animal when it was first exposed to sound. Total incidence of convulsive behavior increased from age 13 days to a peak at 18 or 20 days and declined thereafter. The highest incidence occurred at a 2 or 3 day conditioning-test interval. A high incidence of maximal audio-conditioned seizures first appear in mice audio-conditioned at 15 days of age and few maximal seizures were observed in mice audio-conditoned after 22 days of age. Approximately 11 percent of the animals tested died. The highest death rate occured in 20 day old subjects with 20% and the 18 day old mice where 15% succumbed. The incidence of death is therefore comparable to that observed in Frings and O'Grady mice (Castellion et al., 1965). Death occurred only after a maximal seizure in non-drug treated individuals. Furthermore, death risk was not related simply to age or incidence of maximal seizures as death risk was higher with a 1 or a 5 day condition-test interval than an interval of 2 or 3 days (Table 7).

The time latency (from start of bell) to the onset of tonus revealed that a conditioning-test interval of more than 3 days produced latencies that are usually longer and rather irregular. However, the latency to either component at a 2 or 3 days interval was found to possess a

Table 7. Relationship between Convulsions and Death Risk

Maximal Seizure %	Death Risk after Maximal Seizure[a]	Death Risk after Total Convulsion[b]	Condition-test Interval	Age (days) when Conditioned
7	7.1	3.3	1	24
7	.71	.12	1	15
8	6.3	3.1	5	15
8	2.5	1.1	4	13
11	2.0	.69	5	24
13	3.9	1.9	5	20
19	3.2	2.5	1	20
23	.83	.41	2	22
26	.27	.11	1	18
32	.53	.30	2	15
35	.71	.58	4	18
47	.58	.47	3	18
55	.22	.16	3	15
57	.35	.23	2	18
65	.39	.29	3	20
68	.37	.26	2	20

Groups not exhibiting any maximal seizures or death have been omitted.
a. deaths (%)/maximal seizures (%);
b. deaths (%)/total incidence of convulsions (%).

remarkably small variance. For example, a group of forty mice, 20 day old (test age) and a 2 day condition-test interval, had a latency to wild running of 6.6± .3 seconds (S. E.) and a latency to tonus of 12.9± .7 seconds. Convulsive latencies were distributed in a bimodal fashion much as has been reported for inbred seizure susceptible strains (Fuller and Smith, 1953). However, unlike the susceptible strains, the small but persistant portion of slow latencies (over 30 seconds) were predominately of the clonic type. Only in a few instances did a second running seizure occur, and this only rarely resulted in a maximal seizure. It should be noted that susceptibility to sound-induced seizures could persist for several weeks if the animals were exposed to the bell repeatedly at 2 day intervals (Table 8). Furthermore, a third exposure to the bell at 30 days of age also produced convulsions if the animals had a convulsion during the second exposure. The fact that seizure susceptibility persists if the animal experiences a convulsion indicates the possibility that audio-sensitivity and seizure susceptibility may reflect separate mechanisms.

A conditioning stimulus of 10 seconds or one of 60 seconds duration produced little difference in subsequent seizure incidence. However, if the duration of the conditioning stimulus were increased to 48 hours and the mice were tested after an additional 48 hours had elapsed, none of the animals convulsed (Table 9). Testing these subjects

Table 8. Effect of Age and Repeated Sound Stimulation on Seizure Pattern of CF#1 Mice. Fifty-eight mice 18 days of age were subjected to 60 seconds of sound. The stimulus was repeated every 2 days for 8 trials and again at 40 and 60 days of age.

	CONVULSIVE RESPONSE (%)			
AGE (days)	Clonus	Maximal Seizure	Death	Total
18	0	0	0	0
20	33	50	14	93
22	28	47	12	84
24	42	42	14	92
26	38	25	8	68
28	33	15	0	52
30	15	9	6	27
32	19	3	0	22
34	22	3	0	25
40	10	3	0	17
60	7	0	0	7

Table 9. Effect of Duration of the Conditioning-stimuli upon Seizure Incidence in DBA/J and CF#1 Mice. The second exposure was presented two days after the conditioning stimuli. (a. Third exposure 2 additional days later.)

AGE (days) when Conditioned	DURATION of Conditioning Stimuli	CONVULSIONS (%) Second exposure 2 days later	NUMBER of Animals
		CF#1	
18	10 seconds	84	26
18	60 seconds	88	181
18	48 hours	0	23
		0[a]	23
		DBA/J	
10	200 hours	100	18

a third time; did not result in seizures. It is believed that the mice were not deaf as they demonstrated an auricular startle response. Furthermore, mice of the DBA/2J strain, which had been subjected to continuous sound since they were 10 days of age responded with a maximal seizure provided the conditioning bell had been turned off for over an hour prior to the test stimuli. Mice less than 12 days of age do not exhibit audiogenic seizures. No seizures were observed in any of the mice during the period of continuous sound. Apparently this difference in adaptation reflects an additional difference between genetically susceptible and non-susceptible strains.

DISCUSSION

Mice are considered genetically susceptible to audiogenic seizures if they exhibit a clonic-tonic seizure when tested only once (Vicari, 1947; Fuller et al., 1950). The practice of specifying the first trial is not always observed and may clarify a portion of the controversial literature concerning sound induced convulsions. Previous auditory stimulation was found to be absolutely essential for the development of convulsions in CF#1 mice. Susceptibility to sound-induced seizures in this strain was found to be markedly influenced by the condition-test interval (number of days between the initial and subsequent exposure to sound), the duration of the initial exposure

and age. In view of the many physiological and biochemical differences, reported by several investigators, between strains of mice genetically susceptible to audiogenic seizures (first trial) and nonsusceptible strains; this age and condition-test interval dependent convulsive phenomenon was designated as an Audio-conditioned Convulsive Response (ACCR). Additional justification for such terminology, if necessary, is not difficult in that prestimulation by sound is known to enhance or reduce susceptibility depending upon the temporal parameters of the treatment (Ginsburg, 1963; Fuller and Wimer, 1966). Furthermore, Bevan (1955) in the review of the literature added "The classical priming method of physiology has shown the number of animals convulsing vary with the duration of the conditioning stimulus and the conditioning-test interval." Generally both the duration of the conditioning stimulus and condition-test interval have been short, being only a few seconds (Fuller, et al., 1953; Bevan, 1955; and Frings et al., 1951). Also these terms would enhance clarity in the discussion concerning the study of critical time limits, in terms of the intertrial interval, different trial, periodicity, and age. The audio-conditioning stimuli is "neutral" (does not eliciate a convulsion) and is rather specific to sound. Furthermore, the literature concerning increment changes in responsiveness to a particular stimulus is meagre (Hinde, 1966).

Mice have an acute sense of hearing. The sensitivity to high frequency sound attains adult values by the 14th day (Mikealin and Ruben, 1965) and declines rapidly after 50 days of age (Ralls, 1967). However, the ability of CF#1 mice to respond to sound with a maximal seizure persists for an extremely brief period. Maximal seizures were observed in few subjects under 17 or over 25-days of age. The seizure incidence is apparently dependent on the condition-test interval and the age when audio-conditioned rather than the age when tested. It is interesting to note that the first full tonic-clonic audiogenic seizure pattern in genetically susceptible mice (simulated only once) occurred in 17 day old O'Grady mice; 16 day old Frings mice (Castellion et al., 1965); 17 day old DBA/1 (Frings et al., 1951) or DBA/2 mice (Hamburg and Vicari, 1960) and reached a maximum in all strains by age 22 days.

The induced audio-sensitivity, as measured by seizure susceptibility in CF#1 mice, was possibly the result of a maturation process. Immature animals are notoriously more sensitive to a wide variety of environmental factors than are mature mammals (Done, 1966; Nyhan and Lampert, 1965). The transition from the newborn state to that of the adult is not an abrupt physiological event in that some organs retain some degree of immaturity well into the postnatal period (Fox, 1965). This period is characterized by rapid growth and involves the maturation of several interlocking

systems. Numerous factors may overwhelm the homeostatic mechanism of the immature animal and disrupt the time-dependent systems essential for an orderly maturation (Yaffe and Back, 1966). The initial exposure to the intense auditory stimuli may be disrupting to neurological maturation. However, several reports indicate that the mouse brain approaches anatomical maturity (Miale and Sidman, 1961; Haddara, 1956), chemical maturity (Himwich, 1962), histological maturity (Kobayashi et al., 1963), and physiological maturity (Fox, 1965; Castellion et al., 1965; Ferngren, 1965; Millichap, 1957; Clark and Sarkaria, 1958) when the mouse is approximately 15 to 17 days of age. Perhaps the susceptibility to audio-conditioned convulsions might be a correlate of development and maintenance of neurophysiological and neurochemical processes, such as blood brain barrier, carbonic anhydrase activity, brain electrolytes, mobilization phenomena, etc.

It is our conclusion that the Audio-Conditoned Convulsion has possible utility in maturation studies, or to study various physiological and perhaps psychological factors affectors affecting the nervous system. The phenomenon has potential research value because proper selection of age, conditioning, and the condition-test interval produces seizures that are reproducible and a predictable incidence and severity.

NEUROPHYSIOLOGICAL CHARACTERISTICS OF THE ACCR

In the previous chapter of the investigation a procedure that dramatically increases seizure susceptibility in a strain of mice generally considered to be resistant to sound induced convulsions has been described. Seizure susceptibility was found to be markedly influenced by age, prior auditory-conditioning and especially by the interval in days between the initial stimulus and subsequent exposure to sound. This response has been designated the Audio-conditioned Convulsive Response (ACCR) to distinguish it from the audiogenic seizure in strains of mice that are genetically susceptible.

The present chapter represents an attempt to interpret, in terms of brain mechanisms, the alteration in response to a battery of well standardized, seizure evoking procedures after a single auditory conditioning. Inasmuch as there is a reasonable neurophysiological explanation for seizures evoked by electrical and chemical methods (Swinyard et al., 1963) it is anticipated that underlying neurophysiological mechanism of the audio-conditioning stimuli might be revealed. The ontogensis of seizure susceptibility to several different stimuli in a single strain of mice may also be of some value to interpret certain observations not clearly understood at present.

METHOD

Young CF#1 mice were raised to the desired age as described in the General Procedure. The litters were randomly divided and assigned into groups. They were audio-conditioned in the manner described in the General Procedure. Additional mice of the same age, weight and not subjected to audio-conditioning (and therefore not susceptible to sound induced convulsions) were employed as control animals.

Changes in seizure threshold were evaluated at various intervals after induced audio-sensitivity by two electroshock (maximal electroshock and low-frequency electroshock) and one chemoshock (pentylenetrazol infusion) procedures. Maximal electroshock seizure (MES) threshold was determined by the intensity of stimulus required to evoke a maximal seizure (hindleg tonic extension) in 50% of mice using a Grass S4B stimulator (0.1 msec. pulses; 100 pulses per sec.; and 0.3 seconds stimulus duration; corneal electrodes; as described by Toman, 1955). Alteration in MES pattern and latency were measured to the nearest tenth second with an electric clock. The end points employed for both electrical and sound induced seizures were those suggested for mice by Fink and Swinyard (1959). Minimal, or low-frequency electroshock seizure (lfES) threshold was determined by the intensity of stimulus (Grass stimulator:

0.2 msec.; 6 pulses per second; 3 seconds duration; ear clip electrodes) required to evoke a minimal seizure ("stun response" and 3 seconds of persistant clonic activity) in 50% of mice (Brown et al., 1953). Since even brief restraint and handling may effect seizure threshold care was exercised to measure seizure threshold after the electrodes had been attached for 30 seconds and the animal had begun to move about. The data obtained from studies on electrical seizure thresholds were statistically analyzed by the method of Litchfield and Wilcoxon (1949).

Pentylenetetrazol seizure threshold (PST) was determined by a timed continuous intravenous infusion method. The procedure employed was essentially identical to that employed by Bastian et al., (1959) except that in the younger animals continuous infusion of the convulsant agent solution was by the intraperitoneal route rather than the usual intravenous administration. The solutions were infused at a rate of .006 ml/sec by means of a constant-infusion apparatus (Harvard Model 975) until two end points were observed. The first end point consisted of 3 seconds of persistent clonus, and the second consisted of tonic-extension. The data is expressed as average infusion time and the standard error of the mean was calculated.

Irrespective of the stimulus employed to evoke a maximal convulsion, the overt manifestations are qualitatively the same (Swinyard, et al., 1963). The pattern

of a maximal seizure can therefore be divided into three principal phases: tonic flexion, tonic extension and terminal clonus. When sound or a chemical stimulus initiates the convulsion, preliminary pretonic activity is observed. The significance of the duration of terminal clonus following the tonic phase is unknown, and therefore it was not recorded. The tonic-flexion and tonic extension component of a maximal seizure are distinct events and their latency can be measured accurately.

RESULTS:

The pattern of maximal seizures induced by electrical, chemical and sound stimuli in 20 day old CF#1 mice are shown in Table 10.

The data in this table is in good agreement with that reported by Swinyard (1963, 1963b); the duration and pattern of maximal seizures at a given age are remarkably constant and relatively independent of the stimulus employed. Convulsions induced by intraperitoneal infusion of pentylenetetrazol progress through four stages in a manner analogous to that of intravenous administration that is; initial twitch, clonus, tonus and death (Bastian et al., 1959; Fingl and McQuarrie, 1960). The time of onset is not clear-cut for the first stage. The next two stages can be accurately observed and timed. The time of onset of persistent clonus is taken as a measure of

Table 10. Maximal Seizure Pattern in 20 day old Mice.

Type of Convulsion	Duration of Seizure Components (seconds).		
	Pre-tonic	Tonic-Flexion	Tonic Extension
Electro-shock	a	1.6[b]	17.0
Pentylene-tetrazol	6.8[c]	1.8	20.4
Audio-Con-ditioned	3.0[c,d]	1.8	15.1

a) component too brief to measure.

b) Includes latency.

c) Includes period from first generalized clonic to tonic-flexion.

d) Preceded by 6.6 +.7 (standard error) seconds latency and 3.2 $\pm$.2 seconds running.

pentylenetetrazol seizure threshold (PST). The endpoints, PST, and tonic extension are differentially affected by increasing postnatal age forming a basis for determination of drug distribution and postnatal maturation of the brain.

The maximal audio-conditioned convulsion is similar to audiogenic seizures reported in O'Grady mice (Castellion, et al. 1965). The maximal ACCR has a mean latency of 6.6 seconds in 20 day old mice tested at the two day-condition test interval. The initial latency ended with an explosive burst of wild running, which continued for 3-5 seconds. Abrupt cessation of the running episode occurs when the mouse appears to leap into the air and falls into a period of clonus followed by the abrupt onset of tonic flexion. This period of clonus is often not observed and the mice lie on their side for 1-3 seconds before the onset of the tonic flexion phase.

The pretonic phase following supramaximal electroshock was extremely brief. In contrast the pretonic phase in both pentylenetetrazol and sound induced convulsions lasts several seconds and are characterized by distinct components. The tonic phase of maximal seizures induced by all of the three stimuli was characterized by a brief initial flexion and subsequent prolonged extension of the hindlegs. Occasionally, after sound stimuli a young mouse will not display an extension component because the hind feet are caught in the front feet. However, if freed, the

hind legs do proceed into tonic extension. The mean duration of the hindleg tonic-extensor component of the three types of seizures evoked in 20 day old CF#1 mice resemble each other closely and lasted from 15 to 21 seconds. A period of terminal clonus followed the tonic phase of electroshock and sound induced seizures. Although terminal clonus in adult mice is absent after maximal seizures induced by pentylenetetrazol it is frequently observed in younger mice. A cataleptic state follows terminal clonus of sound induced convulsions, and occasionally electroshock in younger mice. This cataleptic state has been observed to persist for as long as 3-5 minutes. Behavior in the postconvulsive phase is very interesting in that several different motor phenomena including "epileptoid furor" are observed.

Recurrent seizures are not known to occur subsequent to termination of the electroshock stimuli. Recurrent convulsions are frequent following pentylenetetrazol and a second tonic extension usually results in death. Recurrent seizures after audio-conditioned convulsions are not commonly observed and are mainly clonic in nature. However, the following morning several mice may be found dead, especially if positive pressure resuscitation to restore respiration after the convulsion had been used. It is not known if death was the result of a recurrent seizure. If the auditory stimulus is continued, seizures

of a running or clonic nature, but rarely tonic, occur subsequent to the initial maximal seizure.

A difference in mortality rate after a maximal seizure induced by the three stimuli was observed. Adult CF#1 mice seldom die following MES, however 6 percent of mice under 30 days of age die. Twenty percent of the mice tested in the ACCR died after a maximal seizure; whereas death occurs in almost all the mice injected with pentylenetetrazol.

The duration of the components of electrical and chemoshock seizures changed considerably with age. Postnatal brain development is characterized by changes in the time pattern of the MES as shown in Figure 3. The duration of extension decreased until about 60 days of age. The duration of tonic flexion increased from 1.6 seconds at 22 days of age to 1.9 seconds at 30 days of age. Duration of flexion and ability to sustain tonic extension are considered measures of seizure intensity (Tedeschi, 1956; Woodbury, et al., 1952). The maximal electroshock seizure threshold did not change after 20 days of age. However, the ratio of tonic-extension/tonic flexion decreased sharply from 20 to 30 days of age as shown in Figure 4. The low frequency electroshock (lfES) threshold markedly decreased from 20 to 30 days of age (Figure 5). Mice younger than 15 days of age exhibit a different pattern of response to low frequency electroshock and their

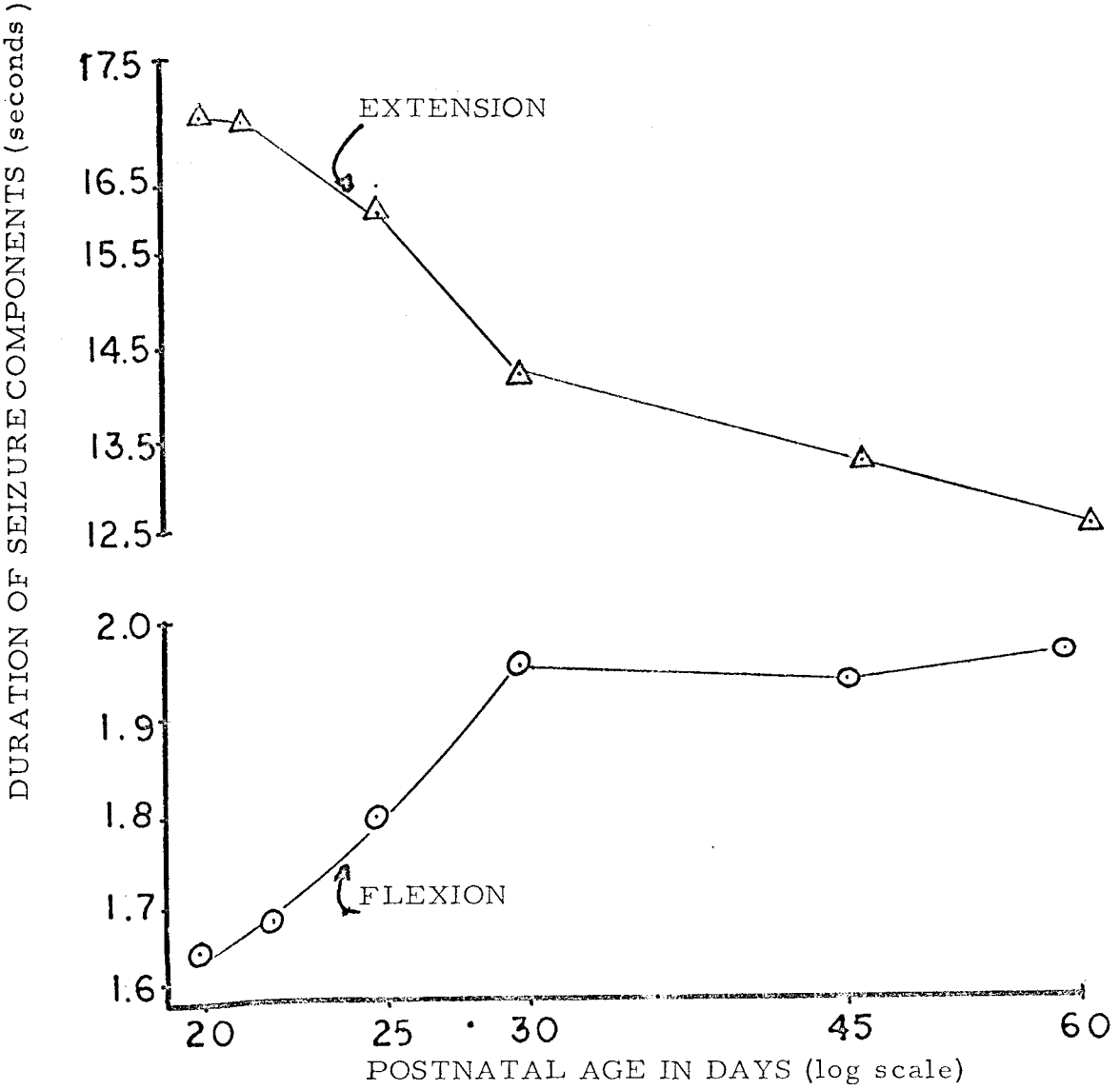

Figure 3. Effect of Age upon Duration of Maximal Electroshock Seizure Components.

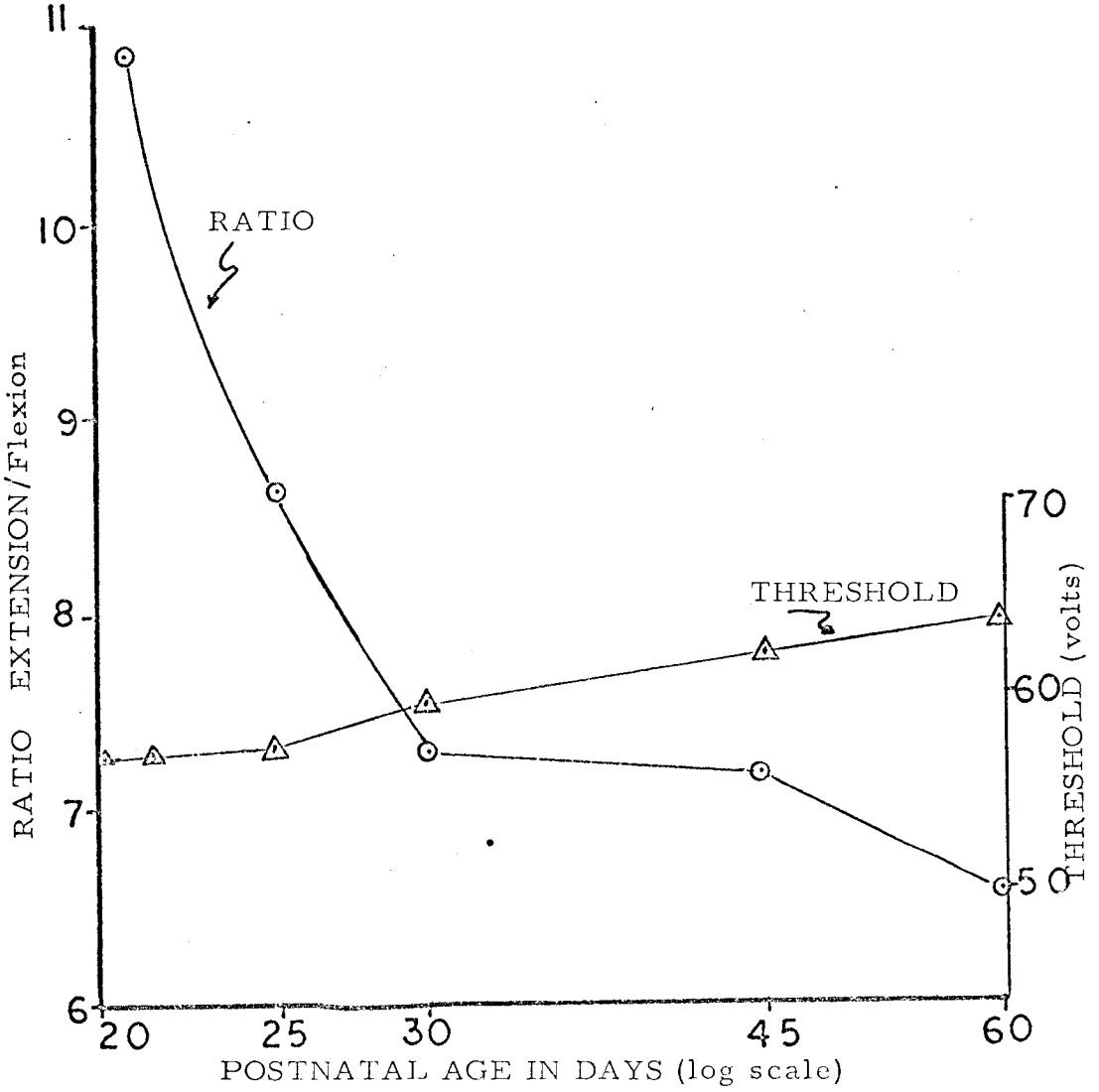

Figure 4. Effect of Age upon Maximal Electroshock Seizure Threshold and Ratio of Extension/Flexion Components.

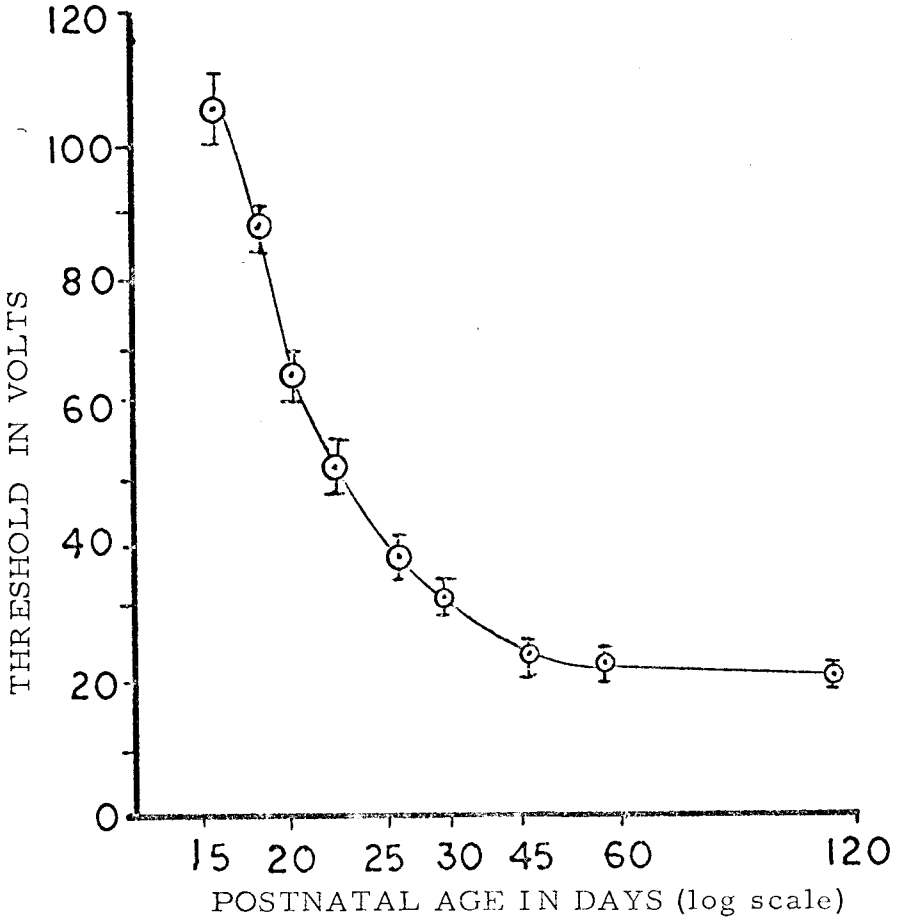

Figure 5. Effect of Age upon Low Frequency Electroshock Seizure Threshold (95% confidence limits)

threshold was not determined. At about day 45 the low frequency seizure threshold apparently reaches the adult level.

The ratio of maximal/clonic pentylenetetrazol seizure threshold decreased from 15 to 24 days of age (Figure 6). In the mouse, hindleg-tonic seizure patterns with pentylenetetrazol do not appear before day 12. However, after 5 days of age, mice do display tonus of the front legs. Since variability in weight may alter the ratio of maximal/clonic pentylenetetrazol seizure the data is calculated on a weight basis in Figure 7. The same relation holds for body weight as for age; this is not unexpected since the animals gain weight as they grow older.

Seizure Threshold Alteration by a single auditory stimulus.

In view of the fact that susceptibility to audio-conditioned convulsions decreased sharply after the 20th day, the effect of a single auditory trial upon the threshold for pentylenetetrazol; maximal and the low frequency electroshock seizures might give some insight into the mechanism of the facilitated audio-sensitivity. The effects of a single 60 second sound stimulation (audio-conditioning) upon threshold and duration of components of the maximal electroshock seizure are shown in Table 11. The single auditory stress did not significantly effect maximal electroshock seizure threshold or pattern. Since no difference in the seizure threshold or pattern were obtained between

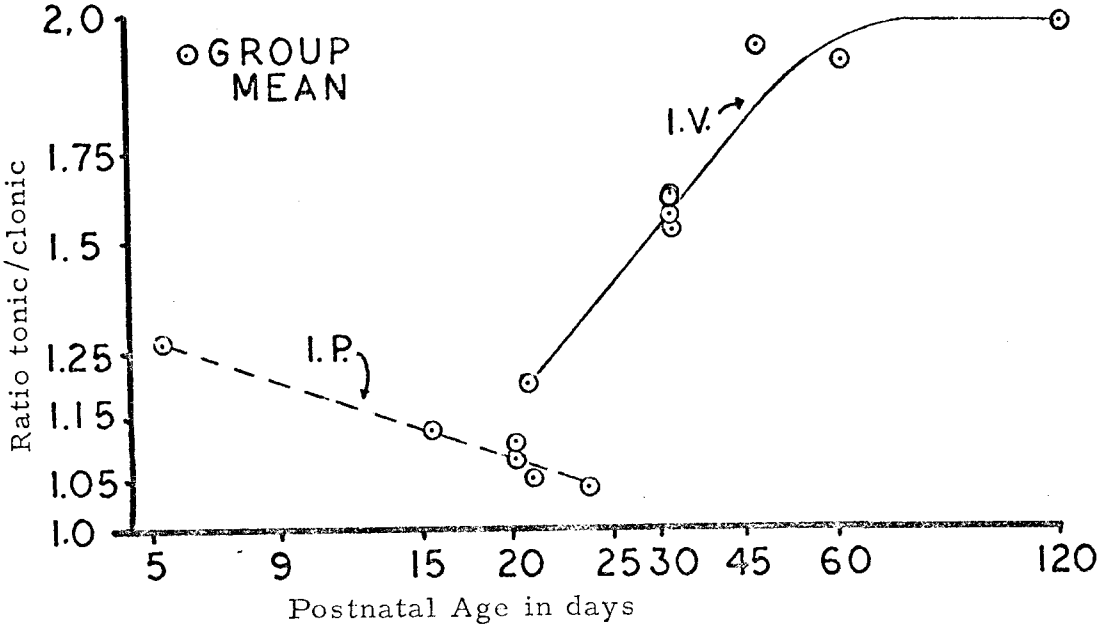

Figure 6. Effect of Age upon tonic/clonic Seizure Ratio for Pentylenetetrazol Threshold (log-log scale).

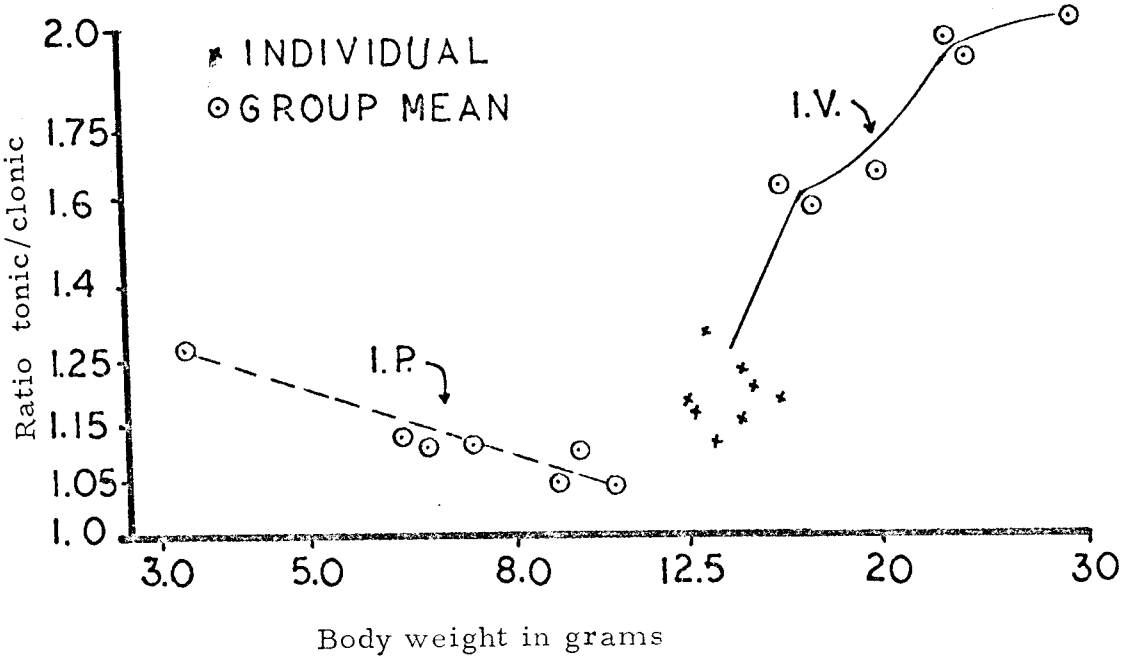

Figure 7. Effect of Body weight upon tonic/clonic Seizure Ratio for Pentylenetetrazol Threshold (log-log scale).

Table 11. Effect of Age and Conditioning Upon Threshold and Duration of Components of Maximal Electroshock Seizure

	POSTNATAL AGE IN DAYS			
20	33	25	30	45
	MAXIMAL ELECTROSHOCK SEIZURE THRESHOLD[a]			
Bell	59(56-62)	58(56-61)	60(57-63)	60(58-62)
----	57(55-60)	57(54-60)	60(58-62)	62(59-65)
	DURATION OF MAXIMAL ELECTROSHOCK COMPONENTS[b]			
	FLEXION[c]			
Bell	1.7 ±.04	1.71±.04	1.78±.05	1.91±.05
----	1.59±.07	1.79±.05	1.94±.07	1.85±.01
	EXTENSION			
Bell	17.47±.25	16.32±.24*	15.25±.27**	12.71±.34
----	16.97±.25	15.69±.21	14.30±.16	13.44±.33
	RATIO EXTENSION/FLEXION			
Bell	10.28	9.54	8.57	6.65
----	10.67	8.76	7.37	7.26

a) threshold in volts, value in parenthesis represent 95% fiducial limits.
b) time in seconds ± standard error.
c) includes latency.
d) average group 42 animals.
* significantly different than control ($P \leq .05$; ** $P \leq .01$).

mice given only one maximal electroshock and those young mice shocked at an interval of several days, it appears that maturation of maximal electroshock seizure pattern was not altered by more than one electroshock. However, the alteration in the duration of extension among sound pre-treated mice suggest that previous electroshock effected subsequent seizure pattern in these mice. Since the mice show a transitory audio-sensitivity that lasts only a few days the alteration in electroshock pattern observed 7 to 12 days after the prime may be the result of a decreased rate of neurological maturation produced by the sound, or by interaction of sound and previous electroshock convulsion.

In Table 12 it may be seen that the thresholds for lfES in the mice treated with sound at 18 days of age was significantly lower than control animals until 30 days of age. Mice first subjected to the bell at 25 days of age do not develop maximal ACCR, nor does audio-conditioning of 25 days old mice significantly alter lfES threshold. Also lfEF thresholds determined at any given age are not altered by previous determinations.

The effects of a single 60 second bell sound on pentylenetetrazol seizure threshold are shown in Table 13. Two days after audio-conditioning the threshold for both clonic and tonic seizure was significantly longer than the controls, by the fourth day the thresholds for

Table 12. Effect of Age and Conditioning Upon Low Frequency Electroshock Seizure Threshold

Mean Threshold (volts) and 95% confidence limits					
POSTNATAL AGE IN DAYS[c]					
18	20	22	25	30	45
Bell[a]	52* (50-55)	38* (36-40)	31* (29-33)	29* (28-31)	25 (24-27)
87[a] (84-90)	66 (63-70)	49 (46-52)	39 (36-42)	34 (32-36	25 (23-28)
-----[a]	63 (60-67)	48 (46-51)	40 (37-43)	34 (32-37)	25 (23-27)
-----	-----	53 (51-55)	40 (38-43)	32 (30-34)	25 (23-27)
-----	-----	-----	Bell[b]	34 (32-36)	25 (23-27)
-----	-----	-----	----[b]	34 (32-37)	25 (23-27)
----	----	-----	-----	-----	25 (23-27)

a) First three groups run concurrently
b) Groups run concurrently
c) Average group 31 ± 2 mice.
* Significantly different than untreated controls ($P < .05$)

Table 13. Effect and Age and Conditioning Upon Threshold[a] for Pentylenetetrazol Seizure Components.

Postnatal Age in days	Conditioning	Pentylenetetrazol conc.	route	No. tested	First Clonic Jerk	Generalized Clonic Seizure	Maximal tonic-Clonic Seizure	Ratio tonic/clonic
20	Bell day 18	1%	IP	14	51.8±1.0*	56.1±.9**	62.9±.9*	1.12
20	none	1%	IP	11	48.5±.8	52.3±.9	59.7±1.1	1.15
22	Bell day 19	.5%	IP	9	71.5±4.2	77.5±3.8	83.1±8*	1.08
22	none	.5%	IP	10	76.2±3.5	81.1±3.3	99.2±2.9	1.14
24	Bell day 18	1%	IP	12	45.3±.7***	48.2±.8***	58.8±1.6	1.22*
24	none	1%	IP	9	56.0±1.2	59.8±1.1	62.8±1.0	1.07
20	Bell day 18	1%	IV	4	17.0±1.3	18.2±.1.4	23.0±1.7	1.24
20	none	1%	IV	4	14.9±.6	16.9±.9	19.9±2.2	1.21
30	Bell day 18	.5%	IV	10	24.6±.7	28.3±.9	38.3±1.0	1.35
30	none	.5%	IV	9	26.0±1.3	27.6±1.1	-----[b]	
40	Bell day 18 Bell day 20 Bell day 30	.5%	IV	11	-----[b]	30.8±.7	59.6±3.7	1.94
45	none	.5%	IV	9	-----[b]	30.3±1.9	59.9±3.2	1.98

a) Timed threshold of components in seconds (mean ± standard error).
b) determination not made.
* Significantly different than control: * P < .05; ** P < .01; *** P < .001.

tonic seizures were decreased and remained low until after the 6th day. The mice were not given pentylenetetrazol more than once. The tonic/clonic seizure ratio was increased 6 days after the audio-conditioning.

Since the single auditory conditioning has pronounced effects upon growth of weanling mice, even though no overt seizure occurs, the PST in mg/kg body weight is illustrated in Table 14. It took significantly greater quantities of pentylenetetrazol to induce clonic and also tonic seizures among the audio-conditioned groups until 12 days after the prime.

Mice raised on enriched diets in order to increase body size to facilitate intravenous injection showed similar results. Such mice subjected to a bell a second time at 20 days of age displayed 90% ACCR susceptibility; and a third exposure at 30 days of age produced 42% convulsions. Despite their decreased body weight at 40 days of age their pentylenetetrazol seizure threshold was the same as the controls. It should be noted that once an observable convulsion has been elicited by sound the susceptibility to ACCR persists for a prolonged period. This suggests that to accurately assess a causal relationship between convulsive activity and changes in neurophysiological or neurochemical processes all secondary changes due to a previous convulsion must be partitioned.

Table 14. Effect of Weight, Age and Conditioning Upon Threshold[a] for Pentylenetetrazol Seizure Components.

Postnatal Age in days	Conditioning	Pentylenetetrazol conc.	route	No. tested	Body Weight in grams	Generalized Clonic Seizure	Maximal Tonic-clonic Seizure
20	Bell day 18	1%	IP	14	5.9±.2	583.6±9.3***	654.1±9.4***
20	none	1%	IP	11	6.3±.2	505.6±9.0	577.6±10.3
22	Bell day 18	.5%	IP	9	7.6±.3***	311.5±.3***	334.1±15.5*
22	none	.5%	IP	10	10.3±.2	239.4±9.7	293.0±8.6
24	Bell day 18	1%	IP	12	6.9±.2***	423.6±.7**	516.7±12.2***
	none	1%	IP	9	9.3±.3	391.0±.7.2	410.1±6.8
20	Bell day 18	1%	IV	4	12.6±.3	88.6±6.8	111.3±8.3
30	none	1%	IV	4	13.3±.6	77.4±4.1	91.3±10.1
30	Bell day 18	.5%	IV	10	14.9±.5	57.9±1.8	78.2±2.1
30	none	.5%	IV	9	15.8±.4	53.2±2.2	----[a]
40	Bell day 18 Bell day 20 Bell day 30	.5%	IV	12	20.2±.5	46.6±1.0	90.2±5.6
45	none	.5%	IV	9	22.0±.5	42.0±2.7	83.0±4.4

a) Threshold in mg/Kg body weight (mean ± standard error).
b) determination not made.
* Significantly different than control: * P < .05; ** P < .01; *** P < .001.

Discussion

The studies presented herein on the effect of postnatal maturation of seizure pattern and threshold indicate several important differences between maximal seizures induced by the three stimuli --chemoshock, electroshock, and audio-conditioned convulsion. The ability of CF#1 mice to respond to sound with a maximal seizure persists for only an extremely brief period. Maximal audio-conditioned seizures first appeared in 18 day old mice and ability to respond to the second sound with a maximal seizure was present only in few individuals over 23 days of age (data in preceding chapter). However, maximal electroshock seizures (MES) first appear in CF#1 mice by 16 days of age (Castellion et al., 1965) and the ability to exhibit a maximal seizure persists throughout life. Development patterns of maximal chemoshock (pentylenetetrazol) convulsions in mice are noted after 12 days postnatal age and like maximal electroshock susceptibility persists throughout life.

A difference in mortality rate among 20 day old CF#1 mice after maximal convulsion, was observed. Adult CF#1 mice seldom die following a maximal electroshock seizure, however 6 percent of the mice under 30 days of age died. Mice over 15 days of age did not die more frequently than

30 day old mice. Twenty percent of the mice exhibiting an audio-conditioned convulsion (ACCR) die, whereas death occurs in almost all the mice injected with pentylenetetrazol. A somewhat higher, although not significant incidence of death after electroshock occurred in mice that were pretreated with sound.

Heim (1963) suggested that the postnatal brain development is characterized by specific responses to electroshock and that correlation exists between the response pattern and developmental changes. Indeed the postnatal responses of the rat to electroshock stimulation have been used to reflect changes in neurophysiologic development by prenatal exogenous stimuli (Heim, 1963; Vernadakis, 1963, 1965, 1966, and 1967).

In mice seizure threshold for maximal electroshock does not change with increasing postnatal age, whereas both low frequency electroshock (lfES) and pentylenetrazol seizure threshold decrease markedly between 15 and 30 days of age. These thresholds appear useful to evaluate the effect of postnatal exogenous stimuli, such as sound. The data suggests that lfES threshold and PST are perhaps measurements of a similar neuronal mechanism. However, the single auditory stimulus enhances the apparent maturation process for lfES threshold, but it retards the development of PST.

The maximal electroshock and maximal pentylenetetrazol seizure threshold apparently reflect different neuronal processes. This maximal chemoshock threshold does change after 15 days of age while the MES is not significantly altered with increasing postnatal age. Furthermore the single sound stimuli that produces suceptibility to sound induced convulsions markedly affected maximal pentylenetetrazol threshold but did not affect MES threshold. The MES and the ACCR would appear to involve different supraspinal mechanisms, since the stimulus that facilitates audiosensitivity did not alter maximal electro-shock seizure threshold or pattern. This suggestion is supported by the fact that supramaximal electroshock in 18 day old mice does not result in susceptibility to sound induced convulsions. Moreover, the maximal electroshock technic does not appear to lend itself to measurement of subtle differences in seizure threshold or seizure spread induced by exogenous stimuli postnally applied.

Comparison of the ontogenesis of seizure susceptibility in audiogenic seizure susceptible (first trial) strains of mice and ACCR (second trial spaced at an interval of days) indicates additional differences between the maximal seizures induced by sound. The ability of CF#1 mice to respond to sound with a maximal seizure persists for an extremely brief period and very little seizure activity is observed on the first exposure to sound. Only a very few

individuals under 18 or over 25 days of age responded to the second sound stimulus with a maximal seizure. The occurence of a seizure is apparently dependent on the age the mouse is first exposed to the bell and the conditioning-test interval rather than the age when tested. Mice audio-conditioned at 12 days of age or less do not exhibit any type of seizure upon the second exposure in sound, regardless of the conditioning-test interval. A peak incidence of maximal seizures occurs at a 2 or 3 day condition-test interval. A high incidence of maximal ACCR first appear in mice audio-conditioned at 15 days of age and the response is rarely maximal in mice primed after 22 days of age.

It is interesting to note that the first tonic-clonic audiogenic seizure (first trial strains) patterns in mice stimulated only once occurs in 17 day old O'Grady mice, 16 day old Frings mice (Castellion, 1965); 17 day old DBA/1 (Frings, 1951) or DBA/2 mice (Hamburg and Vicari, 1960) and reaches a maximum incidence in all strains by day 22. This suggests that maturation of the central nervous system in audiogenic seizure susceptible (first trial) and nonsusceptible strains possess a remarkably similar time course. However, 90 to 100% of the DBA/2 mice have maximal seizures but only 50 to 60% of the CF#1 mice respond maximally to a 95-100 decibel stimulus. The lower incidence of maximal seizures may reflect differences in adaptation or inhibitory processes, rather than threshold. This suggestion is

supported by the observation that testing a known maximal responder at the same time with an audio-conditioned individual resulted in 90% maximal seizures in CF#1 mice. The inherent difference in adaptation in the two strains was further demonstrated by the fact that the DBA/2 strain did not become seizure resistant after chronic exposure to intense sound whereas CF#1 mice did become resistant (Iturrian and Fink, 1968b).

There is no significant difference in the electroshock seizure threshold among animals that do and those which do not exhibit convulsion of the audiogenic seizure susceptible strains of O'Grady, Frings (Swinyard et al., 1963); DBA/2 (Hamburg and Vicari, 1960); or selectively bred stocks of Swiss Webster mice (Hamburg and Essman, 1963). CF#1 mice that are audio-conditioned, and therefore seizure susceptible also do not have an altered threshold for maximal electroshock seizure. However audio-conditioning had profound long terms effects on low frequency electroshock (lfES) threshold. Comparable studies have not been performed in audiogenic seizure susceptible strains, so no comparison of the time pattern effects can be made.

Castellion (1965) reported that the development of the audiogenic seizure pattern is not altered by repeated stimulation, but Frings (1953) suggested that mice tested daily exhibit differences in age distribution of susceptibility. It should be noted that susceptibility to ACCR could persist

for several weeks if the animals were exposed to the bell repeatedly at two day intervals. The fact that seizure susceptibility persists if the animals experiences a convulsion indicates the possibility that audio-sensitivity and seizure susceptibility reflect different intensities of the same mechanism or separate processes acting on a common efferent system. This suggests that to accurately assess a causal relationship between convulsive activity and changes in neurophysiological or neurochemical processes all secondary changes due to a previous convulsion should be partitioned.

The main components of a complete convulsion, modified from Bures (1963); Servit (1963); and Swinyard (1963), initiated by any external stimulus are:

1) The afferent link--
processes evoked in the oscillator (or analyzer) by the epileptogenic stimulus;

2) The mediating link--
mechanisms responsible for propagation of the seizure discharge from the oscillator;

and 3) The efferent link--
mechanisms of the generalized seizure.

The concept of an "oscillator" was proposed (Toman and Taylor, 1952; Woodbury and Esplin, 1959) to call attention to the fact that measurement of seizure threshold involves more than a simple estimation of stimulus required for initial excitation of the neurons involved. They assumed

that in order for a minimal seizure to be evoked, it is necessary for a substantial number of neurons to discharge over a finite period of time. This collection of neurons was designated as the "oscillator" to distinguish it from the seizure focus or exogenous stimulus which serves to trigger the oscillator (Swinyard et al., 1963).

If this neuronal pool is excited at a certain frequency (such as by low frequency electroshock) it discharges, but the discharge spread is limited to adjacent areas and only minimal seizures are evoked. Oscillator discharge of higher intensity results in spread of the discharge to other areas of the brain. The spread of the discharge is under the influence of the mediating mechanism. Upon sufficient spread the efferent link is excited with maximum efficiency and the result is a tonic-clonic (maximal) seizure. Therefore, the intensity of the seizure depends on processes in the mediating link.

The concept of "maximal seizure" was based on observations that the components of a tonic extensor convulsion are relatively invariant in character and duration once the threshold value was exceeded (Tedeschi, 1956). It has been concluded that the maximal seizure pattern is virtually an all-or-none phenomenon and neuronal circuits, particularly those of the spinal cord which are maximally active during the tonic extensor seizure (Esplin and Freston, 1960; Swinyard, 1963). The direct stimulation of the spinal cord

has shown that a limit on the motor patterns of a maximal seizure is imposed by spinal reflex mechanisms (Esplin and Freston, 1960). Indeed it has been suggested that the "maximal seizure" does not represent maximal brain discharge but rather maximal spinal activity (Freston and Esplin, 1961). Thus, maximal spinal cord activity and motor movement may occur with a submaximal brain discharge. Since the data presented indicates the pattern of maximal seizures induced by chemoshock; electroshock and sound stimili are remarkably similar at any given age it would appear that all of these stimuli excite the spinal cord maximally. Therefore it would appear that the distinguishing neurophysiological characteristics of the ACCR must primarily involve supraspinal mechanisms.

Bures (1963) and Servet (1963) have attempted to define the neuroanatomical location of the main seizure components at various levels of the central nervous system. However, the "oscillator" and the mediating link are, at present, more in the nature of a concept than a mechanism with a proven neuroanatomical substrate. Also experiments that lead to the reflex theory indicate that the cortical level of the auditory analyzer (auditory oscillator) may play an important role in the mechanism of audiogenic seizures but the lower parts of the auditory pathways are essential for seizure generalization. Swinyard et al., (1963) have stated that the oscillator of audiogenic

seizure-susceptible strains of mice are more sensitive to electrical discharge than is the oscillator of CF#1 mice. However, their data was obtained from two different strains of mice and may reflect inherent genetic differences rather than discharge of the oscillator mechanism. These investigators also found minimal and maximal pentylenetetrazol seizure threshold to be essentially the same in audiogenic seizure mice (O'Grady) and CF#1 controls. Genetic differences in susceptibility to the convulsant effects of pentylenetetrazol has been reported for several strains of mice (Weaver and Kerley, 1962). Furthermore Busnel and Lehman (1961) found an enhanced susceptibility to pentylenetetrazol. Coulombre (1950) has reported differences in electroshock stimulation to be affected not only by strain but also age. This matter most appropriately re-emphasizes the need and importance of specifying in publications not only the mouse strain used but the age as well.

Bures (1963) by surgical or functional ablation of higher centers of the auditory pathway, noted that the essential parts of the mediating mechanism are located at the bulbar or pontine levels. Kesner (1966) has also investigated the effect of lesions if the subcortical areas of audiosensitive rats. The reticular formation of the lower brain stem is probably also an important part of the mediating system (Servet, 1963). It is suggested that the

intensity of a seizure depends on the strength of inhibitory process and that inhibitory stimuli acts through the mediating mechanism. Therefore, it would seem that the superficial difference between the maximal and submaximal sound induced convulsions actually reveals an underlying inhibition or adaptation in the mediating link, in addition to possible differences in threshold. Furthermore, the "maximal seizure" induced by the different stimuli may reflect submaximal brain discharge from different areas or possibly activation of different processes of the mediating mechanism. This assumption may explain the failure of auditory-conditioning to affect MES threshold or pattern. Moreover the EEG during a maximal seizure induced by various methods is different (Servet, 1963).

It is further suggested that changes in the inhibitory process of the mediating link involve a vital role in the ACCR. The brief susceptibility to ACCR may not be a matter of losing sensitivity but rather a matter of the development of a more prominent system such as an inhibitory system. The ACCR may therefore be a functional disorder of the brain due to a temporary weakening of the inhibitory process permitting a wide irradiation of exitation throughout the brain. Servit (1963); Bures (1963) and others have reported that inhibitory processes are probably an important part of the mechanism involved in sound induced seizures. Development of inhibitory systems could

also account for the change in sensitivity to ACCR produced by ageing and the different conditioning-test intervals. However, this suggestion does not account for the observation that audio-conditioning does not alter MES threshold. Nevertheless, the ACCR might provide a useful experimental model for the study of pathophysiological mechanism of the central nervous system.

The reduction of lfES threshold by the auditory-conditioning suggests involvment of a common mechanism. Excellent evidence for a common mechanism is that audio-conditioning of 25 day old mice did not alter the threshold for lfES nor did it result in facilitated audiosensitivity. However, audio-conditioning 18 day mice relate differences in the time sequences for lfES threshold and ACCR. The difference in time patterns was also observed between penytlenetetrazol thresholds and the audio-sensitive period. Pentylenetetrazol seems to act on cortical as well as subcortical structures (Hahn, 1961), the activity in young animals being mainly evoked subcortically (Cahilhac, 1960).

When seizures are produced by different stimuli, numerous factors other than seizure threshold and pattern must be considered. Sackler (1963) has indicated that endocrinologic effects and mobilization phenomena has an important relationship with audiogenic seizures. Millichap's (1957) interesting analysis of postnatal development of seizure patterns implicated not only brain

carbonic anhydrase activity, but also distribution of water and electrolyte concentration of the brain. Plykko and Woodbury (1961) discussed certain biochemical and physiological changes which occur in the rat central nervous system with ageing.

Clark and Sarkaria (1958) reported young mice are more sensitive to a convulsant dye than adult mice because the blood brain barrier was not functionally complete and that the permeability of the blood brain barrier decreases with increased age of the mice. They also reported electroshock increases the permeability of the blood brain barrier. Electroshock, however, was found not to cause sensitivity to ACCR. Different opinions are held on the permeability of the blood-brain barrier during postnatal maturation (Davison and Dobbins, 1966). Furthermore, little is known about the importance of absorption, penetration of the blood-brain barrier and distribution for the convulsant effect of pentylenetetrazol in growing mice. The alteration of both clonic and maximal pentylenetetrazol threshold by the audio-conditioning procedure may indicate alteration in membrane permeability. The permeability of filtering membrances is increased in many abnormal conditions, e.g. by inadequate blood supply, anoxia, or of various toxic agents including certain drugs (Keele, 1965). The suggestion that audio-conditioning may effect the permeability of the immature blood-brain barrier is supported by the

observation that anoxia was unique among non-auditory stimuli in that it eliciated audio-sensitivity. Sound was by far the most important conditioning stimuli as will be discussed in the next chapter.

STUDIES ON THE NEUROPHARMACOLOGICAL CHARACTERISTICS OF THE AUDIO-CONDITIONED CONVULSIVE RESPONSE

We have previously demonstrated a procedure that dramatically increases seizure susceptibility in a strain of mice generally considered to be resistant to sound induced convulsions. Seizure susceptibility was found to be markedly influenced by age, prior auditory-conditioning and especially by the interval in days between the initial stimulus and subsequent exposure to sound. This procedure has been designated the audio-conditioned convulsive response (ACCR) to distinguish it from strains of mice that are genetically susceptible to audiogenic seizures (AGS). Animals are considered genetically susceptible to audiogenic seizures if they exhibit a clonic-tonic seizure when tested only once (Vicari, 1947; Fuller et al., 1950).

The present paper represents an attempt to interpret, in pharmacological terms, the mechanism(s) involved in the development of the ACCR. In addition it was anticipated that this study would suggest laboratory tests which might be used to assay potential agents for treatment of human emotional disturbances and epilepsy since sound induced convulsions have been used to evaluate tranquilizers (Fink and Swinyard, 1959; Plotnikoff, 1963) and anticonvulsants (Swinyard et al., 1963; Plotnikoff, 1963). ACCR is potentially more useful than previous procedures in that a high incidence of convulsions are produced in mice without using

genetically susceptible strains, special diets, chemicals, or surgical manipulation. Furthermore, mice of the CF#1 strain seldom die from the convulsion which is in sharp contrast to most audiogenic seizure-susceptible strains.

METHOD

Mice (CF#1) purchased from Carworth Farms were bred and the offspring were reared to the desired age as described in the General Procedure. The litters were assigned to a treatment by randomized design and were audio-conditioned in a manner described in the General Procedure. Additional mice of the same age, weight and not subjected to audio-conditioning (and therefore not susceptible to sound induced convulsions) were used as control animals. Since the induced audio-sensitivity is affected by environmental noises (Iturrian and Fink, 1968b) the mice were carefully protected from extraneous noise.

Test Stimulus. The mice were individually exposed to sound a second time (test) after the lapse of the most effective conditioning-test interval (number of days between the first and second exposure), which has been found to be 2 or 3 days for 18 day old CF#1 mice. The method of testing is described in the General Procedure. Alterations in seizure pattern and latency were measured to the nearest 1/10 second with an electric timer. The end points employed were those suggested by Fink and Swinyard (1959) and

described in the General Procedure. The data were evaluated as average values and the standard error of the mean was calculated and analyzed for difference using the Student "t" test (Li, 1964). The X^2 test for independence was used to analyze seizure frequency (Siegel, 1956).

The responses of 20 day old mice are similar to that of 18 day old mice. A third exposure to the bell (challenge) was therefore used to test for prolonged drug action and to detect experimental difficulty. All groups not exhibiting at least 50% maximal seizure during the challenge bell were eliminated.

Experimental conditioning-stimulus: Naruse et al., (1960) has reported several different types of labyrinthine stimulation that were effective in inducing convulsions in an inbred strain of mice (ep). These and several other forms of stress were used in an attempt to define the relative specificity of the audio-conditioned convulsion. In these experiments the audio-conditioning stimulus was replaced by postural stimulation or another standard stress. The duration of the stress was limited to about 60 seconds; and the test bell was not presented until 2 days later. The postural stimulation included: gently tossing the mouse in the air 15-20 cm several times; alternating rotation on a metabolic shaker; to-and-fro horizontal swinging; continuous rotation in one direction; and the "seesaw stimulation" described by Naruse (1960). Additional mice were

placed in a desiccator in which the air was replaced by 99% nitrogen until the mouse lost its postural control due to anoxia. Other standard forms of stress included: placing the mouse upon a 1 cm thick film of mercury; dropping the mouse from 10 feet into sawdust (Wilson, 1963); repetitive shock to the feet through a floor grid (Toman et al., 1955); supramaximal electroshock convulsion (.1 msec. pulses, frequency of 100 pulses per sec., 140 volts, and .3 seconds stimulus duration, Grass S4B stimulator with corneal electrodes); minimal low frequency electroshock (.2 msec. pulse, frequency 6 pulses per sec., 100 volts, and 3 seconds stimilus duration thru ear electrodes); hyponatremia, (Swinyard et al., 1952); or incremental doses of metrazol until a hanging convulsion resulted when the mouse was lifted by the tail and jerked (Wilson, 1963).

Some drugs are known to facilitate audiogenic seizures in mice, therefore several drugs were tested to determine if a drug could induce audio-sensitivity.

Drugs have been used in three different manners in this experiment:

1) to replace the initial (audio-conditioning) stimulus;
2) to theoretically protect or enhance the effects of the audio-conditioning;
3) and lastly to protect the animal from the convulsion produced by the test (second bell exposure) stimuli.

All drugs were evaluated over a three day period using the same bell. The drugs were given in aqueous solution, if water soluble, or orally in 1% methylcellulose. Each drug was tested at the time of its peak activity as reported (Fink and Swinyard, 1959) in adult CF#1 mice by a neurotoxicity test. The dose was selected from those reported as effective dosage (over PD_{50}) for suppressing audiogenic seizures (Fink and Swinyard, 1959; Mercier, 1963). For the drug activity studies, groups of 10 or more mice were used. Additional mice of the same age, and weight were used concurrently with all experiments as nondrug treated control animals.

RESULTS

Experimental-conditioning stimulus: The most effective stimulus that produced an audio-sensitivity, as measured by seizure susceptibility two days later, involved audible sound; however anoxia and ether anesthesia also had some effect (Table 15). The mice subjected to postural disequilibrium exhibited slightly enhanced incidence of seizure activity not demonstrated in drug pre-conditioned individuals. The occurence of death upon the second exposure to the bell (4 days after the conditioning procedure) was greatly enhanced if anoxia was the conditioning stimulus as over 50 percent of these animals died. Furthermore, certain drug pretreated (2 days previously) animals died between the first and second exposure to the bell; but none of these animals convulsed during the initial exposure to the bell. Fifty percent of the animals given amphetamine died after being exposed to the initial bell whereas all the group treated with hydroxyzine died. Very little alteration in the seizure pattern and latency was observed if the animals had received conditioning other than sound at 18 days of age (Table 16). Mercury film stress and hyperbaria altered the latency to tonic-flexion component of the response. Ether decreased the duration of running. The pesticide dieldrin decreased the duration of running and of tonic-flexion. Anticholinesterases are known to promote audiogenic seizure susceptibility (Lehman and Busnel, 1963).

Table 15. Effect of Experimental Conditioning on Seizure Susceptibility in CF#1 Mice.

Conditioning-Stimulus[a]	CONVULSIONS (%) (2 days later) RESPONSE: Minimal	Maximal	Death	Total	(N)[c]
Postural					
Fall	0	14	0	14	(7)
Tossing	0	0	0	0	(11)
Mercury film	13	0	0	25	(8)
Water bath	0	9	0	18	(11)
Shaker	0	0	0	14	(14)
Seesaw	0	0	0	0	(7)
Pendulum	0	0	0	7	(14)
Auditory					
Bell (#5)	14	87	14	100	(7)
Drill in metal	10	30	20	50	(10)
Galton Whistle (setting)					
8	0	9	9	27	(11)
16	0	37	0	37	(11)
Air Blast	13	0	0	38	(8)
Other					
Controls (no treatment)	0	0	0	5	(21)
MES	0	0	0	13	(8)
1f Electroshock	0	0	0	18	(11)
Foot shock	0	22	22	22	(9)
Hyperbaria	0	0	0	0	(8)
Hyponatremia	0	0	0	0	(8)
Anoxia	9	18	0	27	(11)
Anoxia	20	20	10	50	(10)
Dieldrin (25 mg/Kg or)[b]	14	14	14	28	(7)
Hydroxyzine (175 mg/Kg. or)	0	0	0	14	(7)
Nicotine (5 mg/Kg. sc)	0	0	0	6	(17)
Ether	11	33	22	45	(9)
Metrazol (hanging seizure)	0	20	10	20	(10)

Table 15 (cont).

The following drug pretreated groups did not exhibit any convulsions upon the first exposure to the bell 2 days later.

Amphetamine	10 mg/Kg (or)[b]	Methacholine	1 mg/Kg (sc)
Chlorpromazine	32 mg/Kg (or)	Histamine	20 mg/Kg (sc)
Desoxycorticosterone	25 mg/Kg (sc)	Physostigmine	.5 mg/Kg (ip)
Dexamethasone	40 mg/Kg (sc)	Reserpine	33 mg/Kg (ip)
Ephedrine	50 mg/Kg (sc)	Succinylcholine	5 mg/Kg (sc)
Epinephrine	2 mg/Kg (sc)		

a) See text for description of conditioning procedure.

b) route of administration
   (or) oral
   (ip) intraperitoneal
   (sc) subcutaneous

c) Number animals tested.

Table 16. Effect of Conditioning on Latency and duration of Seizure Components Upon the 2nd Exposure to the Bell 4 days later.

Experimental Conditioning Procedure[c]	Mean Latency[a] and duration of seizure components			
	Running	Maximal response (initial clonus)	Duration of running	Duration of initial-clonus[d]
Metrazol	6.3±.9	12.2±1.8	3.4±.5	2.2±.7
Mercury Film stress	12.0±1.5*	19.8±2.6*	3.3±1	3.7±1.3
Swim stress	6.8±.6	3.15.1±1.7	3.2±3	2.9±.1
Fall	6.6±1.1	14.2±.7	3.5±.8	3.2±.6
Anoxia	8.0±1.2	12.4±2.0	3.5±.4	2.6±.2
Anoxia	6.3±.5	10.2±.6	3.8±.3	3.1±.3
Hyperbaria	13.0±2.1*	15.7±1.6	4.2±.4	3.0±.3
Ether	7.8±1.8	9.1±.7	2.6±.1*	3.0±1.4
Dieldrin	6.2±.5	8.4±.5	2.3±.2*	2,0±.3*
Controls (Bell #5)	6.6±1.0	11.3±1.2	4.1±.3	2.9±.4

a) seconds ± standard error of the mean.
b) deviation significantly different than control ($P < .05$).
c) see methods for description of method of conditioning.
d) includes duration of tonic flexion.
* significantly different from the control, * $P < .05$.

Theoretically, at least, pharmacological protection from the effects of the initial exposure to the bell could be afforded by several different drugs. The pharmacodynamic mechanism of the effective drugs in turn might elucidate the mechanism(s) of the facilitated audiosensitivity. Consequently a prototype of several different drug classes were evaluated. The drugs were administered and the mice were audio-conditioned at the time of the drugs peak activity.

Mice that received phenobarbital and audio-conditioning on the same day were found to be exceedingly sensitive as half of them died before testing. No mice given phenobarbital without audio-conditioning died at this dosage. Two mice of the chlordiazepoxide group died and 3 mice in each the promazine and chlorpromazine (32 mg/Kg) group also died before the testing procedure. Thirty-six percent of the mice given reserpine 4 hours previously had maximal sound induced seizures and died when exposed to the initial bell stress. All mice given imipramine had convulsions (drug induced) but only one died. Antihistamines and acetazolamide are known to be very toxic to young animals (Lee, 1966; Petty and Karler, 1965). Several mice given tripelennamine (50 mg/Kg) hydroxyzine (175 mg/Kg) and acetazolamide 90 mg/Kg died. No deaths occurred in the remaining groups.

After a lapse of two days the mice were individually exposed to the second bell. It was assumed that had the drug been effective and if the mice were protected from the effects of the initial sound, no convulsions would occur when the second stimuli was presented. As shown in Table 17, chlordiazepoxide was the only drug that significantly altered seizure incidence at the dosage tested. Chlordiazepoxide, meprobamate, imipramine, methyldopa and edrophonium significantly lowered the incidence of maximal seizures. Edrophonium, however, was effective even if the initial bell preceeded the drug administration by 30 minutes. Acetazolamide and tripelennamine (50 mg/Kg) also decreased the incidence of maximal seizures, however, the dosage exceeded the LD_{50} for young mice.

Some drugs effected the seizure pattern. Tonic-flexion was almost always followed by tonic-extension among the controls, but was observed alone in most tripellenamine treated mice and in some meprobamate, imipramine, amitryptyline, dihydroergotamine and ephedrine pretreated mice.

Some drugs apparently increased the severity of the convulsion as several drug pretreated animals died following the seizure. Indeed phenobarbital pretreated mice died after a clonic convulsion; an event that was never observed in control animals.

The latency to the various components of the audio-conditoned convulsion was altered by several of the drugs

Table 17. Effect of Selected Drugs on the Audio-Conditioning of 18 Day Old CF#1 Mice at the 2 Day Condition-Test Interval.

DRUG	DOSE[a] mg/KG (route)[c]	INTERVAL[b] (hours)	CONVULSIONS (%) (2 days later) Minimal Response	Maximal Response	Death Response	Total Response	(N)[d]
Chlorpro-mazine	8(or)	1.5	0	100	50	100	(4)
	32(or)	1.5	67*	33	0	100	(6)
Promazine	47(ip)	1	44	33	11	78	(9)
Chlordia-zepoxide	25(ip)	2	30	30*	10	60*	(10)
Pheno-barbital	8(ip)	3	20	80	80**	100	(5)
Diphenyl-hydantoin	8(ip)	3	0	89	45*	100	(9)
Acetazola-mide	90(or)	2	38	38	38	88	(8)
Mepro-bamate	40(or)	.5	50*	29*	14	93	(14)
Insulin	6 units (sc)	2	22	56	11	78	(9)
Imipramine	50 (ip)	1	42	33*	25	100	(12)
Amitryp-tyline	5(ip)	2	27	67	7	93	(15)
Dihydro-ergo-tamine	1(sc)	2	45	45	22	89	(9)
Morphine	10(sc)	1.5	7	72	7	93	(14)
Ephedrine	50(sc)	2	45	45	0	100	(9)
Methyldopa	250(sc)	2	53*	27*	0	87	(15)
	500(sc)	2	57*	29*	14	87	(7)

(Continued)

Table 17. (cont).

DRUG	DOSE[a] mg/KG (route)[c]	INTERVAL[b] (hours)	CONVULSIONS (%) (2 days later) RESPONSE				(N)[d]
			Minimal	Maximal	Death	Total	
Edrophonium	1(ip)	.25	38	31*	8	77	(13)
	2(ip)	.25	29	14*	0	87	(7)
Trimethadione	300(or)	2	0	100	22	100	(9)
Tripelennamine	10(ip)	2	55*	18*	0	91	(11)
	50(ip)	2	67	33	33	100	(6)
Reserpine	33(or)	4	0	72	14	86	(7)
Controls (bell#9)		-	22±6[e]	68±9	11±8	93±4	(44)

STATISTICAL ANALYSIS OF THE RESPONSE

	Minimal	Maximal	Death	Total
Sum (249)	78	125	35	222
$\bar{y}$	.324	.502	.141	.892
df	22	22	22	22
SST	10.03	14.22	5.98	2.05
SSE	.219	.250	.121	.097
X^2	45.8**	56.9**	49.5**	21.2

a) dose abolishes tonic extension in approximately 75% of audiogenic seizure susceptible mice.
b) interval between drug administration and audio-conditioning.
c) (or) oral administration
(ip) intraperitoneal administration
(sc) subcutaneous administration
d) Number animals tested
e) ± Standard error of the mean
* significantly different than control, *P < .05; **P < .01.

that had been administered two days previously (Table 18). Edrophonium increased the latency to the running and the minimal seizure components. The lower dosage of tripelennamine and methyldopa increased the latency to running as did chlordiazepoxide and amitryptyline. Several of the drugs affected the latency to the minimal component of response but few minimal responses occurred in the control group and their latency was variable. Promazine and amitryptyline increased the latency to either component of the maximal response. Phenobarbital decreased the latency to either component. Chlorpromazine also increased the duration of flexion. The reserpine pretreatment markedly decreased the duration of tonic-flexion. The variance of the latency of various components was found to be unequal among the drug pretreated groups, therefore the "pooled estimator" of the variance was not used. The "derived Student t" was obtained by the Method of Li (1964, p. 143). The data for seizure latency of the remaining groups is in the appendix.

When the interval between premedication and the testing procedure was three days none of the drugs tested effectively altered seizure incidence. (Table 19) However, trimethadione decreased the incidence of maximal seizures and chlorpromazine and chlordiazepoxide increased the occurence of fatal convulsions.

Table 18. Effect of Selected Drugs on Seizure Latency at the 2 day Condition-Test Interval

Drug	Dose (mg/Kg)[c]	Mean Latency[a] of seizure components: Running	Initial clonus	Tonic extension	Duration of initial clonus[d]
Chlorpromazine	(32)	5.6±.9	7.5±.5	12.3±.3	4.8±.2*
Promazine	(47)	18.6±5.8[b]	15.7±5**	18.9±1.0*	3.7±1.2
Chlordiazepoxide	(25)	14.4±2.4[b]*	20.4±5.3	21.8±3.8	4.0±1.3
Amitryptyline	(5)	12.0±1.9[b]*	20.6±3.2[b]*	19.8±1.6*	3.5±.3
Phenobarbital	(8)	5.1±1.1	6.3±.8*	8.5±1.2*	2.2±.4
Diphenylhydantoin	(8)	6.2±.6	9.7±.8	11.7±.9*	2.0±.3*
Methyldopa	(250)	11.3±1.0**	15.8±1.8	19.1±2.6	3.5±.3
Edrophronium	(1)	14.6±2.6[b]*	13.6±2.2	18.7±3.3	2.9±.6
	(2)	17.5±2.7[b]*	(19.7)	----	----
Tripelennamine	(10)	15.8±3.2[b]*	14.7±2.8[b]	18.3±2.6	2.7±.3
Reserpine	(33)	8.7±.9	12.7±1.0	13.9±.5	1.2±.1[b]**
Controls (Bell#9)		7.6±.3	11.±.4	14.3±.7	3.1±.3

a) seconds ± standard error of the mean
b) deviation significantly different than control group ($P < .05$).
c) see Table 17 for the route of administration
d) includes duration of tonic-flexion
* significantly different than control mean, * $P < .05$; ** $P < .01$.

Table 19. Effect of Selected Drugs on the Audio-Conditioning of 18 Day Old CF#1 Mice at the 3 day Condition-Test Interval.

DRUG	DOSE[a] mg/KG	Interval[b] (hours)	CONVULSIONS (%) (3 days later) Minimal	Maximal	Death	Total	(N)[d]
			RESPONSE				
Chlorpromazine	32(or)	1.5	10	80	50*	90	(10)
Chlordiazepoxide	25 (ip)	2	22	67	67**	89	(9)
Atropine	4 (ip)	2	7	67	20	93	(15)
Trimethadione	300 (ip)	2	6	32*	13	68	(16)
Edrophonium	25 (sc)	.2	0	80	0	80	(5)
Epinephrine	1 (sc)	1	33	44	0	100	(9)
Nicotine	5 (sc)	2	29	43	14	86	(7)
Controls (bell#9)	-----	-----	17	67	0	83	(12)

a) dose abolishes tonic extension in approximately 75% of audiogenic seizure susceptible strains of mice.
b) Interval between drug administration and audio-conditioning
c) route of administration
   (or) oral
   (ip) intraperitoneal
   (sc) subcutaneous
d) Number animals tested.
* significantly different than the control, * P < .05; ** P < .01.

Table 19a. Statistical Analysis of the Effects of Drugs at the 3 Day Condition-Test Interval

	RESPONSE			
	Minimal	Maximal	Death	Total
Sum(83)	12	48	17	71
= y	.145	.578	.205	.855
df	7	7	7	7
SST	.843	2.47	4.01	.781
SSE	.124	2.44	1.63	.124
x^2	6.82	10.12	24.63*	6.32

Chlorpromazine, chlordiazepoxide, and atropine pretreated groups had decreased latency to the running component; while epinephrine produced a prolonged latency. (Table 20). The latency to extension was shortened in the chlordiazepoxide pretreated mice.

The convulsion produced upon the second exposure to the bell might be useful to evaluate potential anticonvulsants and anti-anxiety drugs much as the audiogenic seizure has been used. Drugs known to suppress tonic-extensor component of the audiogenic seizure blocked the occurence of a maximal seizure when administered to CF#1 mice that had previously been audio-conditioned. (Table 21). Sodium bromide, however, increased the incidence of fatal convulsions. The dose of chlorpromazine (45 mg/Kg) that effectively reduced the incidence of running seizures produced extreme ataxia. In addition the mice exhibited over 80 percent seizure activity two days later instead of the 7% expected if the mice were not subjected to sound during the 5 day condition-test interval. The other drugs did not modify total incidence of convulsive behavior. Trimethadione and sodium bromide prolonged the latency of running. (Table 22). The duration of running was increased by trimethadione treatment, however this reflects the occurence of several running episodes before the occurence of a minimal seizure. The control mice exhibiting a minimal seizure also had a significantly longer duration

Table 20. Effect of Selected Drugs on the Latency to Seizure Components at the 3 Day Condition-Test Interval.

DRUG	(Dose mg/Kg)	MEAN Latency[a] of Seizure Components 3 days later. Running	Maximal Initial clonus	Maximal Extension	Duration initial clonus[d]
Chlorpromazine	(32)	5.5±.4**	8.9±.5**	12.4±1.1	3.4±.6
Chlordiazepoxide	(25)	5.5±.6**	8.0±5**	10.1±.4[b]*	2.2±.1
Atropine	(4)	6.7±.5*	23.0±6.0[b]	25.7±5.4	3.9_.3
Trimethadione	(300)	8.2±.5	11.2±.5	14.2±.5	3,9±.2
Edrophonium	(25)	7.8±1.7[b]	9.2±1.2	12.7±.8	2.8+.1
Epinephrine	(1)	14.4±2.8[b]	14.9±2.0[b]	17.0±3.0	2.7±.4
Nicotine	(5)	9.0±1.3[b]	8.8±1.2	12.0±1.2	3.2±.2
Control (Bell #9)		8.5±.6	12.3±.5	16.2±1.8	3.8±.8

a) seconds ± standard error of the mean.
b) deviation significantly different than control ($P < .05$).
c) see Table 19 for route of administration.
d) includes duration of tonic-flexion.
* significantly different than control, * $P < .05$; ** $P < .01$.

Table 21. Effect of Selected Drugs on the Convulsions Induced in Audio-Conditioned CF#1 Mice.

DRUG	Dose[a] (mg/Kg)[c]	interval[b] (hours)	CONVULSIONS (%) Minimal	Maximal	Death	Total	(N)[d]
Trimethadione	300 (ip)	2	36	18**	9	82	(11)
Sodium Bromide	200 (sc)	1	23	46	38*	92	(13)
Chlorpromazine	32 (or)	1.5	83**	0**	0	83	(6)
	45 (or)	1.5	7	0**	0	14**	(14)
Diphenylhydantoin	8 (or)	3	50	0**	0	75	(8)
Phenobarbital	8 (or)	3	80**	10**	10	100	(10)
Control (Bell #9)	-----		22	68	11	93	(44)

a) dose abolishes tonic extension in approximately 75% of audiogenic seizure susceptible strains of mice.
b) Interval between drug administration and exposure to test bell.
c) route of administration
   (or) oral
   (ip) intraperitoneal
   (sc) subcutaneous
d) Number animals tested.
* Significantly different than the control, * $P < .05$; ** $P < .01$.

Table 21a. Statistical Analysis of the Effects of Drugs on the Convulsions induced in Audio-Conditioned CF#1 Mice.

	RESPONSE			
	Minimal	Maximal	Death	Total
Sum(106)	34	39	12	84
=				
y	.321	.368	.113	.792
df	6	6	6	6
SST	5.72	9.34	1.32	7.21
SSE	.218	.233	.100	.165
X^2	43.83*	26.25**	40.17**	13.20*

Table 22. Effect of Selected Drugs on the Convulsions Induced in Audio-Conditioned CF#1 Mice.

Drug	(Dose mg/Kg)[b]	Mean Latency[a] of seizure components		Duration of running
		Running	Minimal	
Trimethadione	(300)	24.5±2.8**	43.2±8.3	21.4±7.5*
Sodium Bromide	(200)	15.6±3.4*	34.0±5.9	7.5±.9
Chlorpromazine	(32)	12.3±4.3	19.4±3.5	6.1±1.3
Diphenyl-hydantoin	(8)	11.1±1.6	14.2±2.0	4.8±1.0
Phenobarbital	(8)	8.3±.8	14.1±.8	5.3+3±.3
Control (Bell#9)		7.6±.3	17.0±2.2	5.8.8±.6[c] (3.9±.2)[d]*

a) seconds ± standard error of the mean.
b) See Table 21 for the route of administration
c) duration of running terminating in a minimal seizure.
d) duration of running terminating in a maximal seizure
* significantly different than the control mean * P < .05
** P < .01

of running than the controls that exhibited maximal seizures.

Discussion

Experimental conditioning: The procedure of facilitating seizure susceptibility by previous conditioning indicates an extreme degree of specificity of the audio-conditioned convulsion for sound. Since some drugs and certain labrinthine stimulation is known to facilitate audiogenic seizures in mice, it was essential to determine the relative specificity of the conditioning procedure for sound.

The exposure of mice to an aversive environment such as postural disequilibrium, foot shock, etc. is known to increase brain excitability in mice (Swinyard et al., 1963 b) and to produce profound metabolic changes in the brain (Naruse et al., 1960). However, no experimental conditioning procedure was found, other than sound, that produced a dramatic increase in seizure susceptibility. Anoxia and ether anesthesia however, did have some effect. The latter may produce cerebral anoxia due to respiratory depression but is also known to produce auditory aberration (Vandam, 1965). The possibility that anoxia may produce a high susceptibility to sound induced convulsions on a different condition-test time pattern than auditory conditioning has not been investigated.

The slow onset of auditory sensitivity (peak effect at 2-3 days) and the ineffectiveness of non-auditory stimulation appear to rule out participation of adrenal secretions as the mechanism of induced sensitivity. Furthermore, drugs including dexamethasone, desoxycorticosterone, epinephrine and reserpine do not produce audio-sensitivity two days later. Reserpine, however was found to produce audio-sensitivity at the four hour condition-test interval. Reserpine is known to potentiate seizure susceptibility in certain strains of audiogenic susceptible mice (Bielec, 1959; Fink and Swinyard, 1959) and to produce audiogenic seizures in non-seizure-prone rats (Cooke, 1961). Chance (1947) has reported that ephedrine enhanced the sensitivity of mice to sound. It was not found to produce seizure susceptibility in CF#1 mice two days after drug administration. A fortuitous observation occurred, however as a cage of mice, given ephedrine two days previously was accidently dropped, and then audio-conditioned resulting in 80% seizure incidence among the 15 mice. Unfortunately, it has not been possible to reproduce the effect.

Seizure Latency: It is well established that the severity of an experimentally induced seizure, either sensory, chemically, or electrically produced can be determined by the latency of onset, the pattern and duration of the seizure. Most investigators consider a short latency as an indication of increased severity or increased susceptibility

(Fuller et al., 1950; Fuller and Smith; 1953; Frings and Frings, 1953; and others). Woodbury et al., (1952) have established that procedures that increase seizure severity decrease the duration of tonic hindleg flexion. These parameters as well as the type of seizure exhibited have therefore been used to determine severity of the audio-conditioned convulsion.

The convulsive latency of the audiogenic seizure is known to be distributed in a bimodal fashion in some strains (Fuller and Smith, 1953; Fuller and Sjursen, 1967) and it appears to be biomodally distributed in audio-conditioned mice. Despite the tendency for bimodality the mean and standard error of the latency for all components of the seizure in non-drug treated animals was found to be surprisingly constant (see Table 16). The most apparent reason is that the proportion of slow latencies is small and some mice have two running episodes, the one with a short latency, and the second usually resulting in a minimal seizure. This data also supports the observation (Fuller and Sjursen, 1967) that long-latency running attacks abate without developing into a maximal seizure. The experimental conditioning stimuli tested had very little effect upon the latency and seizure pattern produced by the second exposure to the bell stimuli. The postural dis-equilibrium produced by placing the mouse on a mercury film is an interesting exception. This form of dis-equilibrium

has not previously been investigated but apparently produced long lasting effects and warrants further study.

Pharmacological protection from the audio-conditioning: Since previous auditory stimulation is essential for genesis of seizure susceptibility in CF#1 mice, pharmacological protection from audio-conditioning would prevent the development of the convulsion produced by a second exposure to sound. Such protection could result from several different contributing factors; 1) blocking the animals' response to the initial bell, 2) inhibiting the unknown neuronal, endocrine, and/or metabolic processes responsible for the slow development of subsequent audio-sensitivity, 3) affecting hearing and possibly other sensoty organs or, 4) by effecting one of the numerous factors involved in the production of the seizure itself.

Permanent effects on sensory organs or on the seizure processs can be partitioned by disregarding animals that do not respond to the third (challenge) exposure to the bell. Drugs that inhibit the unknown process that slowly (over the next 2-3 days) produces audio-sensitivity should be effective if administration is after audio-conditioning. Therapeutically, useful drugs generally have a relative short (in terms of hours) duration of action which should enhance the separation of drug effect on audio-conditioning and its effect on seizure susceptibility. Consequently,

the general theoretical mechanism of an effective drug should be relatively easy to determine using the ACCR.

Unfortunately, the procedure requires the use of weanling mice. The immature are noted for their pharmacologic differences and these differences are of both quantitative and qualitative nature (Nyhan and Lampert, 1965; Yaffe and Back, 1966; Done, 1966). Some differences in sensitivities of the infant and adult animals to drugs have been explained by differences in the ability to metabolize drugs (Jondorf et al., 1958; Fouts and Adamson, 1959; Catz and Yaffe, 1967), or by differences in drug distribution (Kupferberg and Way, 1963; Domek et al., 1960). However, very little is known about the absorption, penetration of the blood brain barrier and fate of any drug in the prejuvenile (15-26 days of age) mouse. The prejuvenile has surprisingly different responses than just younger or older mice. Perngren (1965) reported absorption and penetration into the brain was most rapid at 3 weeks of age. Catz and Yaffe (1967) found a marked reduction in hexobarbital sleeping time and a sudden increase in hexobarbital degradation by liver homogenates as measured in vitro. They offered no clear explanation of the findings however. Furthermore, there are dramatic behavioral changes in the mice at this age. The prejuvenile period is characterized by hypersensitivity to auditory and visual stimuli

and is frequently called the "jumpy phase" (Fox, 1965; Catz and Yaffe, 1967; Scudder et al., 1967; among others).

The ACCR was also found to be confined to the pre-juvenile period in the CF#1 mice. No drug was tested that effectively protected the mouse from the effects of audio-conditioning. Several drug pretreated groups did exhibit fewer maximal responses and prolonged seizure latencies. However, it is difficult to determine if the apparent decrease in seizure severity is not produced by anticonvulsant or tranquilizer properties of drug remaining in the animal at the time of the second bell. The residual concentration of the drug may be pronounced due to immaturity of the metabolizing and excretory potential or by differences in distribution. The fact that seizure severity was not decreased if 3 instead of 2 days elapsed between drug administration and the testing procedure suggests a residual drug effect. However, this cannot be established with certainty at this time.

The data presented also disclose that drug action is importantly related to time, in days since administration. Phenobarbital given shortly (90 minutes) before an experimentally produced seizure is a potent anticonvulsant (Swinyard et al., 1963). Rumke (1967) reported that when phenobarbital was administered two days prior to a chemoshock seizure, it enhanced the effect of the convulsant. This effect was also observed in the ACCR as phenobarbital,

and diphenylhydantoin pretreated groups exhibited enhanced seizure severity as indicated by increased incidence of fatal convulsions and decreased duration of hindleg tonic-flexion. Chlorpromazine or chlordiazepoxide pretreatment, 3 days previously, also increased the occurrence of fatal convulsions. Rumke (1967) attributed the "proconvulsant effect" to increased microsomal enzyme systems as he failed to demonstrate an altered sensitivity to supramaximal electroshock seizures. Latency of the various components were not reported.

If the audio-conditioned convulsion can be assumed not to involve liver microsomal activity, which would seem logical, it should indicate rather clearly that the "proconvulsant effect" may indeed be of central origin. The previous drug administration may result in increased sensitivity to hypoxia incident to the seizure, and therefore increased fatality. However, such sensitivity to hypoxia would not be expected to effect the duration of the tonic-flexion component. Indeed, reserpine pretreatment shortened the duration of tonic-flexion without increasing the incidence of fatal convulsions. These results, however, do not eliminate the possibility that phenobarbital metabolism produces a long acting convulsant. Therefore, the observed effects of a drug may depend on the point in time at which observations are made, not only in terms of hours but days. The observed "proconvulsant" effect may explain the problem

of convulsions occasionally observed if an epileptic patient abruptly discontinues antiepileptic therapy.

The data presented on the pharmacological protection against the initial exposure to the bell indicated that the audio-sensitivity induced in young CF#1 mice by sound might be useful to assay potential tranquilizers and anticonvulsants. Unfortunately, the use of drugs in analyzing the audio-conditioned convulsion is subjected to considerable difficulties: unknown physiologic mechanism: unknown action, distribution, and metabolism of the drug in the immature mouse. Nevertheless, the effects of various drugs on the audiosensitivity may uncover discrete long term effects and differences between drugs.

Drug control of experimental convulsions: A multitude of pharmacological agents promote or inhibit audiogenic seizures in susceptible strains of mice. In general, the convulsants and parasympathicomimetics promote the seizure, while the anticonvulsants, sedatives, tranquilizers and sympathicomimetics have an inhibiting action upon the onset and/or the severity of the seizure (Lehmann and Busnel, 1963). The convulsion produced by the second exposure to sound of young CF#1 mice is apparently modified by drugs in the same manner as the audiogenic seizure.

The research potential of sound-induced convulsions for investigating drug action on the central nervous system has been limited due to the complexities involved in

maintaining susceptible animals. The ACCR does not possess these difficulties. The convulsions produced are of predictable incidence and severity. The latency to onset, and the duration of the seizure components are remarkedly constant.

Sound induced convulsions have been found to be exceedingly useful in evaluating potential anticonvulsants (Plotnikoff, 1963). Furthermore, although many of the tranquilizers show little or no effect on seizures induced by electroshock or chemoshock, they exert profound inhibitory effects on sound-induced seizures (Plotnikoff, 1963; Lehmann and Busnel, 1963). These results suggest that the convulsion produced in CF#1 mice by previous auditory stimulation may prove to be a useful laboratory method in the search for more active psychopharmacologic drugs. The method warrants consideration because of its simplicity and reliability.

General Discussion

The study of behavioral development has several important as well as fascinating parameters. It is clear from the present investigation that the prejuvenile period (15-26 days of age) of mice is characterized by a multitude of complex interacting maturational changes. The period may be represented as one of overgeneralized responsiveness, a time when neural organization of the central nervous system is integrated to permit adaptation to the environment. It is well known that supranormal stimulation may cause morphological, biochemical and behavioral changes (Himwich, 1962) and consequently may alter the pattern of neurological maturation. The development of audio-sensitivity after exposure to the initial bell therefore may be a disruption in the time-dependent integration of neuronal organization. The delayed behavioral effects of neuronal disruption may reflect the fact that neurologic changes precede ontogenic behavioral ones (Fox, 1965). However, this type of time-space pattern is a novelty in biological systems as the systematic knowledge of living organism is restricted to a time element of minutes, or hours, only rarely to more than one day. Therefore, extensive standardization of all experimental procedures was essential and additional experiments are required to validate any proposal of mechanism(s) for the ACCR.

Nevertheless potentially useful information was obtained, and several interesting prospects have emerged.

The apparent multitude of interlocking-time-dependent systems of postnatal maturation further strengthens the notion that the principles of embryological teratology are relevant to studies in developmental pharmacology. These principles were summarized by Wilson (1965). Generally, the state of development at the time of teratogenic action is an important determinant as it governs which tissues are susceptible to malformation. The effective dosage levels are inseparably related to the age of the animal. During maturation, a chemical may have very severe effects upon the growing animal and produce only mild reaction in an adult.

Environment is known to effect physiologic timing of exogenous origin (Scott, 1962). The changing responsiveness induced by sound to electrical and chemoshock seizures lead one to speculate the probability that the timing of susceptibility varies from one potentially noxious agent to another. This probability is also observed in the "proconvulsant" effect of phenobarbital and possibly the induced audiosensitivity produced by anoxia. Such time-space changes in responsiveness prompt one to consider new domains of research such as temporal toxicology or perhaps temporal pharmacology to clarify these mechanisms.

The investigation may contribute to understanding the basis of controversy in the literature of sound induced convulsions. The importance of specifying the trial when characterizing a rodent population has been emphasized and may clarify a portion of this controversy and the inability of geneticists to designate an audiogenic seizure locus. The recognization that the bell intensity affects susceptibility perhaps should be extended to a critical comparison of nonauditory, as well as frequency characteristics of the stimulus. The relationship between anoxia and sound induced convulsions warrants further investigation. This view has been strengthened by the observation that respiratory arrest and death is not a result of increasing seizure susceptibility. This may explain the apparent low incidence of death in strains that possess a high seizure risk (Fuller and Sjursen, 1967).

The investigation demonstrates a need for more data on the pattern of drug action and metabolism in the prejuvenile mouse before drugs can be effectively used to obtain a profile of ACCR seizure action. Finally, the data re-emphasizes the need for specifying the strain and age of the animal used in the investigation.

Although only a few parameters of neurological development of the mouse were studied, it is clear that ACCR is a simple and reliable test that could serve as an indicator of normal neuro-behavioral development. However, the

multitude of changes that occur emphasize the importance of employing a wide battery of tests in order to determine any potential postnatal teratogenicity of a chemical. The ACCR should be a useful indicator of behavioral teragoteratogenic effects of drugs as Chase (1967) reported 42% of mentally retarded children were epileptic.

SUMMARY AND CONCLUSIONS

Sound produces a high incidence of convulsive behavior in certain strains of mice but others are resistant. However, susceptibility to sound-induced convulsions in a resistant strain (CF#1) was found to be markedly influenced by prior auditory-conditioning, the condition-test interval (number of days between the initial and subsequent exposure to sound), and age. Previous auditory stimulation was found to be absolutely essential for the genesis of convulsions. Incidence in 12 or 45-day old mice was about 5% at any interval. However, 18 day old mice subjected to brief intense auditory stimulation (audio-conditioning) and tested at intervals of 1, 2, 3, 4, or 5 days resulted in a high incidence of convulsions. Clonic-tonic convulsions characterized the seizures at the 2 or 3 day interval; but at 5 days only clonus was seen. Susceptibility to sound-induced seizures persists for a prolonged period if the animals are exposed to the bell repeatedly at 2 day intervals, or if the animal had once experienced a convulsion. Proper selection of age, conditioning, and the condition-test interval produces convulsions that are reproducible and of a predictable incidence and severity.

Mice that had been audio-conditioned were subjected to a battery of standardized, seizure-evoking procedures and the responses interpreted in terms of neurophysiological mechanism. In addition, the effect of maturation on

the development of sound induced, chemoshock and electroshock convulsion threshold and pattern was studied. The data indicate that the pattern of maximal seizures induced by electroshock, pentylenetetrazol and sound are remarkably similar at a given age. It is suggested that the motor pattern in a seizure is limited at the spinal level and that maximal spinal activity can occur with a submaximal brain discharge. The threshold for low frequency electroshock and for minimal pentylenetetrazol decreased with increased age and weight. The low frequency electroshock and minimal pentylenetetrazol threshold are measurements of similar neuronal mechanisms. There was no significant difference in maximal electroshock seizure threshold with aging but duration of the seizure pattern decreased with increased age. Mice that had been audio-conditioned responded to the test stimulus with a maximal seizure provided they were between 18 and 23 days of age. It may not be a matter of losing sensitivity to the initial sound but rather a matter of the development of a more prominent system such as an inhibitory system. An inherent difference in the adaptation of an audiogenic seizure susceptible strain (DBA/2J) and CF#1 mice was demonstrated by the fact that the DBA/2J mice did not become seizure resistant after chronic exposure to intense sound. Audio-conditioning produced profound changes in the ontogenic development of low frequency and chemoshock seizure threshold, but maximal

seizure threshold and pattern were unaffected. Drugs which effectively modify maximal audiogenic seizure pattern, the anticonvulsant and tranquilizers, are also effective in suppresssing audio-conditioned convulsions. No drug negated the effects of audio-conditioning. Anoxia was unique among nonauditory stimuli in that it elicited audio-sensitivity. The audio-sensitized CF#1 mice differ in at least two ways from littermates. First, the oscillator mechanism of the audio-sensitive mice is more easily discharged by electric and chemical stimulation, and second, there is an alteration in the neuro-ontogenic pattern. It was concluded that the Audio-conditioned Convulsive Response has several potential applications, and is a simple but reliable test in assessing the degree of neural maturation of the mouse.

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Appendix

APPENDIX. Effect of Selected Drugs on Seizure Latency at the 2 day condition-test interval with 20 day old mice.

Drug	(Dose mg/Kg)[c]	Mean Latency[a] of seizure components 2 days later. Running	Minimal	Maximal initial clonus	Maximal extension	Duration of initial clonus
Chlorpromazine	(8)	5.1±1.0	------[d]	8.2±1.5	11.8±2.1	3.6±.7
	(32)	5.6±.9	11.0±1.2	7.5±.5	12.3±.3	4.8±.2*
Promazine	(47)	18.6±5.8[b]	36.5±10.1	15.7±.5**	18.9±1.0*	3.7±1.2
Chlordiazepoxide	(25)	14.4±2.4[b]	17.9±2.4	20.4±5.3	21.8±3.8	4.0±1.3
Phenobarbital	(8)	5.1±1.1	21.0	6.3±.8*	8.5±1.2*	2.2±.4
Diphenylhydantion	(8)	6.2±.6	------	9.7±.8	11.7±.9*	2.0±.3*
Acetazolamide	(90)	8.0±.9	28.3±13.5	9.9±1.0	11.9±1.2	2.0±.2*
Meprobamate	(40)	7.1±.7	36.3±7.9	10.8±.7	14.3±.4	3.9±.5
Insulin	6 units/Kg	10.8±1.8[b]	52.1±4.8	19.6±4.5[b]	17.0±2.9	3.6±.5
Imipramine	(50)	16.3±4.3[b]	24.4±9.5	25.4±8.8[b]	32.3±23[b]	3.7±1.4
Amitryptyline	(5)	12.0±1.9[b]*	39.1±13	20.6±3.2[b]*	19.8±1.6*	3.5±.3
Dihydro-ergotamine	(1)	10.8±2.0[b]	49.6±4.5	17.4±4.4[b]	15.8±3.2	2.4±.2

(Continued next page)

Drug	(Dose mg/Kg)[c]	Mean Latency[a] of seizure components 2 days later. Running	Minimal	Maximal initial clonus	Maximal extension	Duration of initial clonus
Morphine	(10)	11.6±1.3[b]	40.2±10.8	17.4±4.3[b]	19.9±5.3[b]	3.3±.3
Ephedrine	(50)	8.9±.9	16.8±5.0	13.7±2.1[b]	15.4±2.2	3.9±.7
Methyldopa	(250)	11.3±1.0[b**]	31.±6.0	15.8±1.8	19.1±2.6	3.5±.3
	(500)	5.9±.8	11.1±.1	9.1±.5	11.8±.6	2.7±.1
Edrophonium	(1)	14.6±2.6[b*]	45.3±4.3*	13.6±2.2	18.7±3.3	2.9±.6
	(2)	17.5±2.7[b**]	50.7±4*	(19.7---	--------	-------
Trimethadione	(300)	6.5±.7	---------	9.8±.7	12.8±.7	2.9±.2
Tripelennamine	(10)	15.8±3.2[b*]	29.5±5.7	14.7±2.8[b]	18.3±2.6	2.7±.3
	(50)	6.8±1.0	11.3±.6	16.2----	----------	--------
Reserpine	(33)	8.7±.9	---------	12.7±1.0	13.9±.5	1.2±.1[b**]
Controls	(Bell #9)	7.6±.3	17.0±3.8	11.1±3.8	14.3±.65	3.13±.27
Sum	---	10.5±.5[b]	29.2±2.2	13.9±.8[b]	15.5±.2	3.0±.2

a) seconds ± standard error of the mean.
b) deviation significantly different than the control group ($P < .05$)
c) see Table 17 for the route of administration.
d) determination not made or missing value.
* significantly different than the control mean * $P < .05$; ** $P < .01$.

Effect of Conditioning on Latency and Seizure Components Upon the 2nd Exposure to the Bell

Experimental Conditioning Procedure[c]	Mean Latency[a] of seizure components 4 days later. Running	Duration of running	Maximal response (initial clonus)	Duration of initial-clonus[d]
MES	11.5±3.8[b]	3.7±1.1	12.2±1.7	2.3±.5
lfES	6.8±.6	4.1±.4	13.7±1.1	2.8±.4
Foot-shock	7.3±.5	3.4±3	13.6±.3	3.0±.3
Metrazol	6.3±.9	3.4±.5	12.2±1.8	2.2±.7
Mercury Film stress	12.0±1.5*	3.3±.1	19.8±2.6*	3.7±1.3
Swim Stress	6.8±.6	3.2±.3	15.1±1.7	2.9±.1
Fall	6.6±1.1	3.5±.8	14.2±.7	3.2±.6
Drill in metal	8.8±.5	3.8±.6	11.5±.5	3.2±.4
Galton Whistle setting 16	8.5±.12	3.0.4*	10.1±1.0	3.0 .5
setting 8	7.8±.7	3.4±.8	10.4±.7	2.7±.4
Alarm clock	8.2±1.0	4,4±.8	12.0±1.3	4.1±1.1
Air blast	6.8±1.0	3.7±.7	14.2±1.1	3.0±.7
Anoxia	8.0±1.2	3.5±.4	12.4±2.0	2.6±.2
Anoxia	6.3±.5	3.8±.3	10.2±.6	3.1±.3
Hyperberia	13.0 2.1*	4.2±.4	15.7±1.6	3.0±.3
Ether	7.8±1.8	2.6±.1*	9.1±.7	3.0±1.4
Amphetamine	6.9 .5	4.3±.6	9.8±1.0	2.2±.2
Dieldrin	6.2±.5	2.3 .2*	8.4±.5	2.0±.3*
Chlorpro-mazine	7.4±1.9	3.8±.2	9.2±.8	4.1±.6
Bell (#5)	6.6±1.0	4.1±.3	11.3±1.2	2.9±.4

a) seconds ± standard error of the mean.
b) deviation significantly different than control ($P < .05$)
c) see methods for description of method of conditioning.
d) includes duration of tonic flexion:
* significantly different from the control, * $P < .05$.