SOUND-INDUCED SEIZURES IN RATS FED AN AMINO-ACID DEFICIENT DIET[1]

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In the past 12 years well over a hundred papers have appeared which have dealt with "audiogenic" seizures. None has succeeded in delineating the basic mechanism or mechanisms involved in these response patterns. Nonetheless, there is accumulating a solid body of data which clearly indicates that changes within the internal environment of the animal subject influence the frequency and severity with which it can be made to convulse. Outstanding among these changes are those associated with generally reduced food intake as well as with deprivation of specific dietary constituents, particularly the B complex vitamins and magnesium (1). Meanwhile the basic metabolic systems are lipid, carbohydrate, and nitrogenous. Common sources of the nitrogenous dietary are cereal grains, such as corn and oats. These, however, do not possess as high a nutritional value as animal protein. For example, zein, the corn protein which constitutes the sole source of the nitrogenous dietary in our present experimental diet, is inadequate for growth or the maintenance of normal body weight, serum protein level, hemoglobin concentration, and red-cell count in rats (3, 6). These adverse physiological effects have provoked speculation concerning possible influences upon the several behavior processes in this species. Unpublished experiments of the first writer have indicated that rats maintained on a rolled oats diet showed wider shifts in activity level than their controls maintained on a standard laboratory diet; also, rats fed a zein diet showed a decrement in their performance of a simple maze habit. These latter also displayed greater irritability or nervousness as indicated by more biting and scratching at the retrace doors, explosive darting in the various alleys, greater distractibility, and resistance to handling. These last observations led to the conjecture that amino-acid deficient animals might also show increased susceptibility to sound-induced convulsions.

METHOD

The general plan of the experiment was as follows: A number of albino rats known to be susceptible to "audiogenic" attacks were subjected to ten exposures to a seizure-provoking stimulus. Two matched groups were then formed on the basis of the proportion of convulsions, running attacks, and nonreaction secured from each animal. One group, the *E* group, was placed on ad libitum feeding of the experimental diet for 34 days. Fourteen days after the start of this regimen a second series of ten exposures was carried out. Following this, the group was returned to free feeding of a standard diet, a third test series being conducted after an interval of two weeks. The second group, the *C* group, was maintained on the standard diet restricted in amount of intake to that of the *E* group. Otherwise, it received the same treatment as the *E* group.

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Population

Thirty-six animals, giving either a running or full convulsive reaction to a 2-min. auditory stimulus, were selected as subjects from a group of 70 drawn from seven litters. All were born within the same week and were approximately 30 days of age at the end of the first ten exposures (Test Sequence I). The *C* group consisted of 9 males and 9 females; the *E* group, of 8 males and 10 females. Three members of the *E* group died before completion of the experiment, and, hence, records on them were eliminated from all statistical manipulations of the data.

Diet

During the period of regulated feeding, the *E* group was provided with unlimited amounts of the following diet: zein, 22 per cent; cornstarch, 53 per cent; butter oil, 11 per cent; yeast extract, 6 per cent; salt mixture, 4 per cent; cod liver oil, 4 per cent. Sufficient tryptophane was added to this to insure a minimum of 3.0 mg. of this constituent per day per animal.

The only source of protein in this diet, with the possible exception of a small amount in the yeast extract, is zein. It, however, is practically devoid of lysine and tryptophane. The amount of lysine in zein is cited as 0.06 per cent (8) as contrasted with 2.6 per cent in dry skim milk and 7.2 per cent in blood meal; estimates of tryptophane content range from 0.03 per cent to 0.09 per cent as contrasted with 0.32 per cent in dried milk (3) and 1.3 per cent in blood meal (4). Rose (7) states that lysine must constitute 1.0 per cent of the rat diet, and tryptophane 0.20 per cent, if members of this species are to grow and develop normally. It is clear, therefore, that the experimental diet is inadequate for these functions. In order to reduce the deficiency to lysine alone, daily supplements of tryptophane were provided as indicated above. Recent unpublished evidence suggests, however, that the diet may be lacking in methionine and other essential amino acids.

It is felt that this diet contains all the constituents necessary for optimum growth with the exception of the amino acids. The salt mixture contains all the minerals known to be required, and the yeast extract and cod liver oil provide both water-soluble and fat-soluble vitamins. There has been some criticism of the use of yeast extract on the basis of lack of knowledge of its constituents. However, when a solution of water-soluble vitamins was substituted for the yeast, no difference in the degree of deficiency was noted.

During this same period of regulation the *C* group was fed powdered Purina Laboratory Chow, restricted in amount to the mean consumption, by weight, of the experimentals on the preceding day. This was done in an attempt to hold constant the intake variable, since the first writer had previously found a markedly reduced intake in amino-acid deficient animals. The water intake of the control group was similarly limited.

Apparatus

The test chamber consisted of a galvanized iron tub with a top diameter of 17 in. and a depth of 10 in. suspended in a metal frame so as to hang free on sides and bottom. This entire unit was enclosed in a cork-lined wooden box topped by a hinged observation window. The entire structure was painted flat black to reduce glare. Sound stimulation was furnished by a 4-in. doorbell mounted so as to project out into the top center of the test chamber. Each animal was tested in a wire mesh cage 9½ in. in diameter and 10 in. deep placed about 1 in. from the sound source. Throughout the experiment the animals were kept in individual cages in a well-lighted, well-ventilated room.

Procedure

Test Sessions

Each animal was exposed to 30 stimulations by the bell. These 30 tests were divided into three test sequences: Test Sequence I, during which the animals were allowed unrestricted

intake of Purina Chow; Test Sequence II, during which dietary controls were in effect; and Test Sequence III, during which the subjects were restored to unregulated feeding. Sequences II and III were each separated from its predecessor by a 14-day period during which the animals were neither handled nor tested. Within each sequence, tests were conducted between seven and eleven on alternate nights. Each animal was weighed at the beginning and end of each sequence.

Testing Procedure

Each subject was tested individually. It was placed in the test cage, the bell was turned on for 2 min., and a comprehensive record of the animal's reactions during the period of stimulation was made. This record included a listing of the various symptoms and the order in which they appeared, as well as a measure of the latency and duration of the running attacks. The length of the latencies was measured from the onset of stimulation to the onset of the running. In most instances there was little difficulty in distinguishing the latter; however, where doubt existed, the time recorded was that at which the subject's behavior had become so violent and erratic as to be judged clearly abnormal.

TABLE 1

MEAN WEIGHT IN GRAMS OF THE EXPERIMENTAL AND CONTROL GROUPS AT THE BEGINNING AND END OF EACH TEST SEQUENCE

	SEQUENCE		
	I	II	III
C (*N* = 18)			
Wt. at start	65	121	175
Wt. at end	122	121	199
E (*N* = 15)			
Wt. at start	60	111	162
Wt. at end	117	109	187

The severity of each response was classified on a four-point scale from no response to severe convulsion. If presentation of the stimulus failed to elicit at least one period of vigorous blind running, the trial was classed as nonreaction (*N*). The running response (*R*) usually began with a startle and crouch followed by backward circling and sidling movements and explosive darting about the cage. This latter culminated in one or more periods of violent running. If running terminated in an epileptoid seizure, the reaction was labeled a convulsive response (*C*). If the convulsion was extremely severe, the response was designated *C'*. In these attacks the animal quickly entered the convulsive stage, usually first displaying extreme tonic rigidity. This latter was usually rapidly replaced by severe clonic movements terminating in a deep coma, during which the animal again became rigid, falling on its side, its ears drawn back against its head, its eyes tightly closed, its forelimbs pulled in against its chest, its hind limbs rigidly extended. This last was followed shortly by an extended period of quiescence and waxy rigidity.

RESULTS AND DISCUSSION

Weight Changes

A striking departure from the usual succession of body weight changes with age is found in the record of both the *E* and *C* groups (see Table 1). No reliable

difference existed between the groups at 30 days (*diff.* = 5; t = .84; df = 31; P = .40), and at the end of Sequence I both had doubled their weights, as expected. After 14 days of controlled feeding, however, this rapid gaining had ceased. The *C* group during this interval failed to show any significant change (*diff.* = 1; t = .35; df = 17; P < .80), a condition attributable to a general state of inanition induced by restriction this group's food intake to that of the *E* group. Meanwhile, for the same period, the *E* group showed a small but reliable drop (*diff.* = 6; t = 3.45; df = 14; P = < .01), a fact which is highly significant in the light of the appreciable gain normally observed for this period. This implies a more severe state of insufficiency than that resulting from simple quantitative control and further suggests, since both groups consumed comparable amounts of food by weight, a more severe level of inanition due to the less efficient utilization of food materials in the absence of lysine and possibly other essential amino acids. This difference in degree of effect is further brought out by a highly suggestive between-groups difference for the beginning of Sequence II (*diff.* = 10; t = 1.62; df = 31; P = < .11). On the other hand, neither group showed a significant change for Sequence II. This implies that during 34 days of restricted feeding, the deficiency in the controls showed no progressive increase in severity, while in the experimentals the greatest effect occurred in the first half of the period of regulation. This, in turn, suggests the possibility of some homeostatic mechanism operating to preserve the animal's biological integrity in the face of the dietary threat.

When returned to ad libitum feeding of Purina Chow, both groups showed the same marked weight gain during the 14-day interval following Sequence II, attaining normal weights for animals of their age. The *C* group gained an average of 54 gm. (t = 24.55; df = 17; P < .001), the *E* group increasing 53 gm. (t = 15.54; df = 14; P = < .001). Both groups showed further significant increases during Sequence III, the *C* group gaining on the average 24 gm. (t = 6.78; df = 17; P = < .001), the *E* group, 25 gm. (t = 7.10; df = 13; P = < .001). These latter changes, however, were only about half as great as those of the 14-day interval between Sequences II and III, and of Sequence I.

This slowing down is probably a function of age, since at 118 days the subjects are entering adulthood. The mean weights for both groups at this point are those of normal 120-day-old animals.

Changes in the Severity of Response

After Sequence I the animal subjects were divided into two matched groups on the basis of the number of *C'*, *C*, *R*, and *N* responses displayed by each subject (see Table 2). The adequacy of this match is indicated by the composite χ^2, 1.11 (df = 2; P = .56) obtained for the categorized Series I responses of the two groups. It is realized that the appropriateness of this application of the χ^2 technique may be challenged since the data do not meet strictly the criterion of independence, i.e., the control data were drawn from ten trials with 18 subjects, rather than one trial with 180 subjects, and the experimental data were similarly collected. The test, however, is believed to be valid since the composite χ^2

for the Sequence II data is highly significant ($\chi^2 = 116.85; df = 2; P = < .001$). The *E* group during the second sequence shows a marked increase in sensitivity, their responses on 60 per cent of the trials being classed in the *C'* category as compared to 13.3 per cent for Sequence I. The frequency of the *R* responses exhibits a commensurate drop. A similar shift toward greater frequency of the more severe responses, though much less pronounced also appears in the *C* group data. This is attributed, as was the *C* group's failure to gain weight during Sequence II, to the reduction in amount of food ingested. The difference in increase further noted between the two groups indicates the effect of differential feeding. It reveals the experimental diet to be less adequate than the Purina Chow, not only for the support of those aspects of normal physical growth reflected by alterations of body weight, but also for the maintenance of a normal resistance to convulsive seizure. This can be accounted for in at least two ways. The omission of lysine and/or other essential amino acids (*a*) effects a general

TABLE 2

DISTRIBUTION OF RESPONSES OF EXPERIMENTAL AND CONTROL GROUPS FOR EACH TEST SEQUENCE

SEQUENCE	GROUP	RESPONSE CATEGORY			
		C'	*C*	*R*	*N*
I	*C*	17	48	109	6
	E	19	41	90	0
II	*C*	13	67	93	7
	E	88	44	18	0
III	*C*	0	35	112	33
	E	8	11	106	25

state of heightened sensitivity within the central nervous system through its effect upon the efficiency with which the various dietary constituents are assimilated, or (*b*) represents in itself, a severe imbalance within some specific metabolic dimension or dimensions, reflected in the lowering of the threshold of a definite convulsive mechanism.

These findings, furthermore, are in apparent conflict with the report of Lacey (5) that susceptible animals display a higher serum protein level than non-susceptible animals. This difference may be reconciled by the suggestion that any deviation from the normal level is accompanied by a shift toward greater sensitivity. The present results are of interest in that they increase the varieties of deficiency associated with increased seizure susceptibility and point to a basic metabolic system worth more of the physiological psychologist's attention.

Re-establishment of free feeding in Sequence III was accompanied by a decrease in the frequency of the more severe attacks. The experimental group gave convulsions in 13 per cent of the trials, as contrasted to 88 per cent in Sequence II; the controls 14 per cent, as contrasted to 44 per cent in Sequence II. Furthermore, the number of convulsions noted for Sequence III drops signifi-

cantly below the Sequence I level. The reason for this is at present not clear; it might reasonably be due to nutritional status, age, amount of previous testing, unknown changes in the stimulus conditions, or interactions among these. Meanwhile the composite χ^2 obtained for Sequence III, 3.30 ($df = 2$; $P = .19$), indicates that a difference in susceptibility between groups, as revealed by the magnitude of the frequencies in the various response categories, no longer exists.

Changes in Response Latency

A simple analysis of variance applied to the mean latency times for the first running attack produced an F-ratio for the between-sequences variance of 13.05 ($df = 2/96$; $P = < .001$). Differences obtaining between groups for any given sequence, and those appearing within either group for any pair of sequences, were evaluated by means of Student's t for the significance of a difference between means of small independent and small related samples, respectively. The between-groups comparison for Sequence I reveals the experimental animals to be reliably faster reactors than their controls ($diff. = 10.45$; $t = 5.12$; $df = 31$; $P = < .001$). This difference, furthermore, is augmented after the period of dietary restriction ($diff. = 13.58$; $t = 3.89$; $df = 31$; $P = < .001$), but disappears with the restoration of free feeding ($diff. = 1.91$; $t = .50$; $df = 31$; $P = < .60$). The diverse factors tentatively advanced to explain the reduction in severity of response noted in both groups for the third sequence may also be proposed to account for this greater similarity in latency in the last series.

TABLE 3

MEAN LATENCY TIMES IN SECONDS FOR FIRST RUNNING ATTACK IN EXPERIMENTAL AND CONTROL GROUPS DURING VARIOUS TEST SEQUENCES

GROUP	SEQUENCE		
	I	II	III
C	37.23	21.47	23.30
E	26.78	7.89	25.31

Changes in latency on successive sequences, meanwhile, clearly demonstrate the results of the dietary treatment (see Table 3). The experimental animals during Sequence II show a reduction in their response time of about 70 per cent, the controls dropping approximately 43 per cent. Again, the greatest shift occurred in the animals on amino-acid restriction. With a return to ad libitum feeding in Sequence III, the *E* group regained their preregulation latency scores, the scores of the controls, in contrast, indicating no reliable change over their Sequence II level. The significance of this discrepancy in the effects of restoration is at present not clear.

SUMMARY

The present experiment was designed to explore possible differences in sensitivity to sound-induced seizures in rats fed a normal and an amino-acid deficient

diet. Thirty-six seizure-susceptible animals were divided into two matched groups according to the proportion of convulsions, running attacks, and nonreactions secured from a series of ten exposures to auditory stimulation. One group was maintained on ad libitum feeding of a diet deficient in lysine and possibly other essential amino acids; the other received portions of a standard laboratory diet comparable in amount to the consumption of the first-mentioned group. During the periods of dietary regulation, both groups were subjected to an additional series of ten exposures. Following this, all animals were placed on unlimited feeding of the standard diet and a final series of ten exposures carried out. The following results were obtained:

1. During the period of dietary regulation, the animals restricted only in amount of intake failed to show any significant change in body weight. Those fed the amino-acid deficient diet showed a slight but significant loss in body weight. After 14 days of rehabilitation with a normal diet, however, both groups of animals had attained weights expected for their age level.

2. During the period of dietary regulation, both groups displayed an increase in the frequency of the more severe types of reaction. Moreover, the increase was greatest in the amino-acid deficient group. After return to normal feeding, both groups exhibited fewer of the more severe seizures than in the initial series.

3. During the period of dietary regulation, both groups showed a decided decrease in the length of the latency period for the first running attack. This drop was most pronounced in the amino-acid deficient animals. After restoration to the standard diet these animals returned to their prerestriction latency level.

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