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Effects of convulsions on grid shock sensitivity of the mouse

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THE EFFECTS OF CONVULSIONS ON GRID SHOCK SENSITIVITY OF THE MOUSE

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Introduction

Convulsions produced by drugs, chemicals, electrical or auditory stimulation have been studied extensively in rats and mice with the primary aims of uncovering the physiological mechanisms of seizures and to observe their behavioral consequences.

At the molar level, Gellhorn, Kessler, and Minatoya (1943) reported that convulsions produced by pentylenetetrazole, insulin coma or electroshock restored experimentally extinguished conditioned responses. Griffiths (1956) also found convulsive agents such as pentylenetetrazole, carbon disulfide, and auditory stimulation effective in reinstating an extinguished conditioned response, but the form of the recovered habit differed depending on the agent. Erikson, Porter, and Stone (1948) demonstrated that electro-shock convulsions impaired the learning and retention of a relatively complex multiple-T alley maze.

At the molecular level, Ginsburg and Huth (1947) detected increased amounts of acetylcholine in the submaxillary glands of mice following audiogenic seizures.

Gellhorn and Safford (1948) found that electroshock convulsions greatly increased the hypoglycemic response in rats. D'Amour and Shaklee (1955) reported an increase in weight of the adrenal glands after repeated audiogenic seizures in rats. Malmo (1957) demonstrated that strong auditory stimulation produces physiological changes in psychoneurotics. While there are many studies reporting physiological changes due to convulsions, relatively little work has been done on the effects of convulsions on central nervous system mechanisms.

Other reports have dealt with alteration of sensory mechanisms by a variety of conditions such as isolation (Melzack & Scott, 1957; Griffiths, 1960), intense stimulation (Griffiths, 1960), and food and water deprivation (Griffiths, 1962). Melzack and Scott (1957) studied the effects of isolation on pain responses in dogs. Dogs isolated from puppyhood to maturity were found to be significantly more tolerant to pain than normally reared littermate controls. Griffiths (1960) subjected one group of rats to "stressful" stimulation produced by light, sound and grid shock on alternate days for two months. Another group experienced isolation for two months. It was reported that both treated groups (isolated and stressed) were significantly more tolerant to grid shock than the

control group. In another study, Griffiths (1962) reported that as deprivation of food and water increased, grid shock tolerance increased. Werboff and Corcoran (1962) found audiogenic seizure-prone rats to be significantly less tolerant to grid shock than seizure-resistant animals. The authors questioned whether the seizure experience or the animals' inherent predisposition to seizures was responsible for the sensory effect.

On the basis of these investigations of alteration of sensory processes by different experiences, the present experiment was designed to investigate the effects of convulsive experiences on grid shock threshold. The following questions were asked: 1. Is grid shock threshold altered by convulsions or by the administration of convulsive agents? 2. Does the nature of the convulsive agent have differential effects on grid shock threshold? 3. Are alterations of grid shock sensitivity related to elapsed time between the convulsions and the post-test grid shock threshold determinations?

Method

Subjects. One hundred forty-five AKR/J♀ x DBA/2J♂ male mice, 20 to 22 days of age at the beginning of each treatment, were used. Twenty-five Ss were rejected for failure to meet experimental criteria (See p. 9). This

hybrid strain (referred to as AKD2F_1/J at The Jackson Laboratory) was used because of its high susceptibility to audiogenic seizure stimuli and low mortality rate. The Ss were obtained from the production colony of The Jackson Laboratory, Bar Harbor, Maine.

Experimental Design. The experimental design was a 2 x 3 x 2 analysis of variance design (Winer, 1962). As can be seen in Table 1, there were two groups (pentylenetetrazole or sound stimulation), three treatments (con-

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Insert Table 1 about here

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vulsive stimulus, subconvulsive stimulus or control), and two time intervals (2 hrs. or 2 days). The pentylenetetrazole group consisted of six subgroups: convulsive stimulus x 2 hrs. (CP1); subconvulsive stimulus x 2 hrs. (SP1); injection control x 2 hrs. (HP1); convulsive stimulus x 2 days (CP2); subconvulsive stimulus x 2 days (SP2); and injection control x 2 days (HP2). The sound stimulation group consisted of six subgroups: convulsive stimulus x 2 hrs. (CS1); subconvulsive stimulus x 2 hrs. (SS1); handling control x 2 hrs. (HS1); convulsive stimulus x 2 days (CS2); subconvulsive stimulus x 2 days (SS2); and handling control x 2 days (HS2).

The experimental paradigm was a test-treatment-retest

sequence for all groups. A simple analysis of variance was computed on pre-test shock threshold scores between all 12 subgroups. As can be seen in Table 2, no significant dif-

Insert Table 2 about here

ferences were found between subgroups. On the basis of this finding, the data were subjected to a 2 x 3 x 2 analysis of variance (Winer, 1962) to test for main effects and interactions.

Apparatus. The apparatus for measuring grid shock sensitivity consisted of a Plexiglas box, 7 1/2 x 7 1/2 x 2 1/2 in. high, with a grid floor. The grids were stainless steel, 1/8 in. in diameter and placed 1/4 in. apart. A Plexiglas circular wall 7 in. in diameter and 1 1/2 in. high was placed within the box. The grid shock was from an AC constant voltage transformer with an output of 115v 60c. The voltage was varied by a variable transformer (Variac Powerstat) with an output from zero to 120 volts which was passed through a step-up transformer, and a shock scrambler. Resistors of 150,000 ohms were placed in series with the animal to compensate for variation in its resistance. Shock intervals of 5 sec. for each Variac reading were controlled by an automatic timer. The shock apparatus is shown schematically in Fig. 1. A vacuum tube voltmeter was used to calibrate the

Insert Fig. 1 about here

voltage from the grids, as shown in Table 3.

Insert Table 3 about here

A sound-deadened fibreboard rectangular box was used when introducing sound stimulation. It had a 1-in. insulation and interior dimension of 11 x 11 x 9 1/2 in. with a removable stainless steel pan, 1 in. deep. The door was provided with a 1/4 in. thick transparent plastic window for observational purposes. A 6-in. doorbell with a sound level reading between 96 and 98 db above 0.0002 dynes/cm.^{2} R.M.S. pressure was used as a sound source. An H. H. Scott Type 410-A sound level meter was used for calibrating sound. A 100 watt bulb was used outside the box as a light source for viewing. According to Shafer and Meyer (1953), light has no effect on audiogenic seizure susceptibility. A photograph of the auditory apparatus is shown in Fig. 2.

Insert Fig. 2 about here

Procedure. Each S was ear-punched between 11:00 A. M. and 1:00 P. M., one day before experimentation. The Ss were housed three to a cage in rooms with regulated temp-

erature from 70° to 74° F., and lights on from 6:00 A.M. to 6:00 P.M. The Ss were weighed before every treatment or shock threshold determination. Room temperature and humidity readings were taken.

Twelve of 15 Ss were randomly assigned to one of 12 subgroups and the remaining three were used as replacements. Pre-test shock thresholds were taken for all 15 Ss. This was followed by a treatment and S was returned to its cage. After 24 hrs., S received a second treatment. This was followed by either a 2-hr. or 2-day time lapse and a post-test shock threshold determination. The testing procedure was repeated on ten different nights using new Ss each time. All testing, whether treatment or shock threshold tests, was conducted at night between 7:00 P.M. and 2:00 A.M. because incidence of audiogenic seizures is higher during the early evening (Reiss, Spain, & Molomut, 1955; Halberg, et al., 1955).

Grid Shock Threshold. The grids of the shock box were wiped with distilled water before each S was tested to insure good contact. Each S was placed in the shock box and received current at a rate of one unit increment (defined as one scale unit Variac reading) per 5 sec. until squeaking was heard. The median of three determinations was the grid shock threshold score. There was a

60 sec. recovery period between each trial. The above procedure was standard throughout the study.

Treatments. Each of 20 Ss of pentylenetetrazole subgroups CP1 and CP2 was given an intraperitoneal (IP) injection of 1% pentylenetetrazole at a dosage of 72 mg./kg. of body weight and placed in a jar for 10 min. or until S convulsed. Each of 20 Ss of subgroups SP1 and SP2 was given an IP injection of 1% pentylenetetrazole at a dosage of 32 mg./kg. of body weight and placed in a jar for 10 min. Each of 20 Ss of subgroups HP1 and HP2 was given an IP injection of distilled water at 50 mg./kg. of body weight and placed in a glass jar for 10 min.

Each of 20 Ss of the sound stimulation subgroups CS1 and CS2 was placed in the auditory apparatus for 60 sec. of exploratory behavior as described by Frings and O'Tousa (1950). Each S was subjected to continuous auditory convulsion-inducing stimulation for 90 sec., as suggested by Frings, Frings and Kivert (1951) and Fuller (1951), or until S convulsed. Each of 20 Ss of subgroups SS1 and SS2 was placed in the auditory apparatus for 60 sec. of exploratory behavior. Each S was subjected to 5 sec. of auditory stimulation followed by 30 sec. of silence, and then 10 sec. of auditory stimulation. Each of 20 Ss of subgroups HS1 and HS2 was placed in the auditory apparatus for 105 sec. with-

out sound stimulation.

Of the 25 Ss which were rejected, four died; two failed to respond to high-level dosage pentylenetetrazole; seven did not convulse to high-level sound stimulation; and 12 had convulsions when subjected to low-level sound stimulation. All rejected Ss were replaced.

Results

A 2 x 3 x 2 analysis of variance was performed on the post-test grid shock threshold scores. Table 4

Insert Table 4 about here

shows there were no significant differences among the main effects or interactions, thus indicating that neither treatment, agent used or time intervals between the last treatment and the post-test grid shock threshold significantly alter grid shock threshold. However, when mean scores were plotted with their 95% confidence intervals, as shown in Fig. 3, the handling control sub-

Insert Fig. 3 about here

group HS1 showed a significantly lower mean grid shock threshold than the subconvulsive stimulus subgroup SS1 when post-test shock threshold was obtained 2 hrs. after the last treatment.

Table 5 shows that the post-test grid shock threshold

Insert Table 5 about here

means are lower than the pre-test grid shock threshold means for the following groups: pentylenetetrazole, 2 hrs.; pentylenetetrazole, 2 days; sound stimulation, 2 hrs.; and sound stimulation, 2 days. Four 2 x 3 analyses of variance were used to determine if there were any significant differences between pre- and post-test grid shock threshold scores. For each analysis, there were two levels (pre-test or post-test) and three treatments (convulsive stimulus, subconvulsive stimulus or control). A summary, as shown in Table 6, shows that post-test grid shock threshold

Insert Table 6 about here

scores are significantly lower than pre-test scores for pentylenetetrazole, 2 hrs. ($F=7.08$, $df=1/54$, $P<.05$), pentylenetetrazole, 2 days ($F=5.12$, $df=1/54$, $P<.05$), sound stimulation, 2 hrs. ($F=7.50$, $df=1/54$, $P<.01$), but not for sound stimulation, 2 days ($F=3.60$, $df=1/54$, $P>.05$). Mean scores with their 95% confidence intervals are shown in Fig. 4. The mean post-test threshold score is signifi-

Insert Fig. 4 about here

cantly lower than the mean pre-test threshold score in the handling control subgroup HS1, the pentylenetetrazole subgroup CP1, and the injection control subgroup HP2.

The possibility that shock threshold was related to humidity, temperature, weight or the time the score was obtained was not supported by the finding of low correlations between pre-test grid shock threshold and humidity ($r=+.305$, $df=118$, $P<.01$), weight ($r=-.278$, $df=118$, $P<.01$), temperature ($r=+.133$, $df=118$, $P>.05$), or the time the score was obtained ($r=-.021$, $df=118$, $P>.05$).

Discussion

The results have demonstrated that convulsion-inducing stimulation, whether pentylenetetrazole or sound, does not significantly alter shock threshold in the $AKD2F_1/J$ hybrid mouse, 2 hrs. or 2 days after the last convulsion.

The results, in general, are not consistent with either Griffiths' (1960) study or Werboff and Corcoran's (1962) study. In Griffiths' study, rats subjected to two months of "stressful" stimulation produced by light, sound and grid shock showed increased tolerance to grid shock. Werboff and Corcoran (1962) reported that administration of two audiogenic seizures produced a decrease in grid shock threshold, but the authors added that the effect may have been due to the inherent predisposition of Ss. The dif-

ference between these studies may be attributed to such factors as: species and genetic differences, the prior experience of the animal, age, stimulus type, and/or the nature of the tests and measurements used.

In Griffiths' (1960) study the "stressor" stimulation is extended over a longer period of time than the present study, which may account for the difference in results. Thompson (1957) has suggested that the effects of prior experiences on subsequent "stress" resistance may be purely quantitative in nature, whereas the modality employed to administer the experience may be relatively unimportant. It is possible that the "stressor" stimulation of the present study was not "stressful" enough to cause a significant effect on the grid shock threshold. In other words, Ss of the present study should have been given more convulsions over a longer period of time. This may be explained by fitting Griffiths' data to the U-shaped relationship model (Fiske & Maddi, 1961), as seen in Fig. 5. The three groups in

Insert Fig. 5 about here

Griffiths' study are equated to three levels of "total impact." "Total impact...is determined by the variation, intensity, and meaningfulness of stimulation from extero-

ceptive, interoceptive, and cerebral sources" (Fiske & Maddi, 1961, p. 30). In the Griffiths study, mild total impact (isolation) requires greater (grid shock) stimulation for arousal; "normal" total impact (control) requires "normal" (grid shock) stimulation for arousal; and excessive total impact (prolonged stressors) once again, requires greater (grid shock) stimulation for arousal.

Assuming that the quantitative nature of "stressful" stimulation is important, it appears that the amount of "stressful" stimulation in the present study is not enough, and the results of the present study should be considered as "normal" total impact.

As shown in Fig. 3, the handling control subgroup HS1 had a significantly lower mean shock threshold than the subconvulsive stimulus subgroup SS1, but the HS1 subgroup was not significantly lower than the convulsive sound stimulation subgroup CS1. At this time, it is difficult to interpret these findings.

Table 6 shows that the mean post-test shock threshold is significantly lower in the pentylenetetrazole, 2-hrs. group; the pentylenetetrazole, 2-day group; and the sound, 2-hrs. group as compared to the pre-test grid shock threshold. The sound, 2-day group shows a decrease, but it is not significant. These findings suggest that we need to

study changes in grid shock threshold over time before we can study the effects of stressors on grid shock sensitivity.

The results of this study make it clear that more work needs to be done in the area of "stressors" altering sensory mechanisms. It is suggested that measures of sensitivity, use of independent variables, and methods of experimental design need to be improved before explanations in this area can be adequately formed. Finally, it is suggested that the underlying neuroanatomical and neurophysiological properties of this phenomenon also need further exploration.

Summary

The present study was concerned with the effects of convulsions on grid shock sensitivity in mice. It was found that convulsions produced by pentylenetetrazole or sound do not significantly alter shock threshold in the AKD2F_1/J$\male$ hybrid mouse, two hours or two days after the last convulsion. However, it was found that grid shock threshold decreases with time. Theoretical interpretations of the results are discussed.

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Table 1

Experimental Design

Group	Treatments	Time Lapse	
		Two hours	Two days
Pentylene-tetrazole	Convulsive	CP1	CP2
	Subconvulsive	SP1	SP2
	Control	HP1	HP2
Sound	Convulsive	CS1	CS2
	Subconvulsive	SS1	SS2
	Control	HS1	HS2

Table 2

A One-way Analysis of Variance of Pre-test Grid Shock Threshold

Source	df	MS	F
Subgroups	11	2.86	.311
Error	108	8.92	----

Table 3

Approximate Calibrated Voltage Steps of Shock Apparatus

Variac reading	Est. voltage at grid	Variac reading	Est. voltage at grid
10	3.3	28	13.2
11	3.8	29	13.8
12	4.3	30	14.4
13	4.8	31	15.0
14	5.1	32	15.5
15	5.5	33	15.8
16	6.0	34	16.3
17	6.6	35	17.0
18	7.3	36	17.8
19	7.8	37	18.5
20	8.6	38	19.0
21	9.1	39	19.5
22	9.6	40	20.0
23	10.2	41	20.5
24	10.9	42	21.2
25	11.2	43	21.8
26	11.8	44	22.5
27	12.4	45	23.2

Table 4

A 2 x 3 x 2 Analysis of Variance of Groups vs. Treatments vs. Time Lapse for Post-test Grid Shock Threshold

Source	df	MS	F
Groups (A)	1	14.07	3.59
Treatments (B)	2	3.36	.82
Time Lapse (C)	1	.54	.13
A x B	2	.48	.12
A x C	1	1.63	.40
B x C	2	7.76	1.84
A x B x C	2	2.61	.64
Error	108	4.09	----

Table 5

Mean Scores for Pre- and Post-test Grid Shock Threshold

Group	Mean Grid Shock Threshold	
	Pre-test	Post-test
Pentylenetetrazole, 2 hrs.	19.30	17.80
Pentylenetetrazole, 2 days	19.03	16.87
Sound, 2 hrs.	18.70	16.97
Sound, 2 days	18.53	17.43

Table 6

Analyses of Variance Used to Determine Differences Between Pre- and Post-test Grid Shock Threshold (A) for Three Treatments (B) for Each Group

	Pentylenetetrazole				Sound			
	Two Hours		Two Days		Two Hours		Two Days	
Source	df	MS	df	MS	df	MS	df	MS
Grid Shock (A)	1	70.42*	1	33.75*	1	45.06**	1	18.15
Treatments (B)	2	3.05	2	1.05	2	2.03	2	4.87
A x B	2	3.32	2	.65	2	7.23	2	2.40
Error	54	9.94	54	6.59	54	6.01	54	5.04

*P .05

**P .01

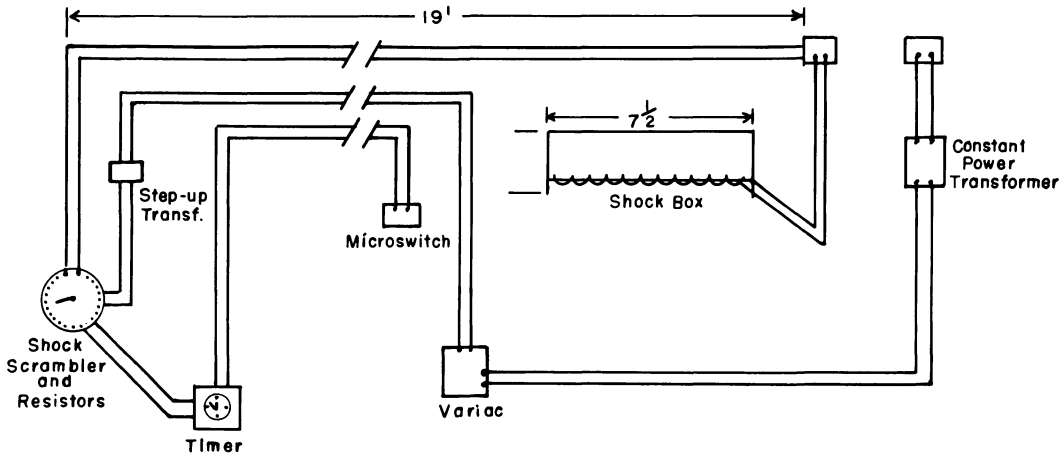

Fig. 1 Apparatus for obtaining grid shock threshold.

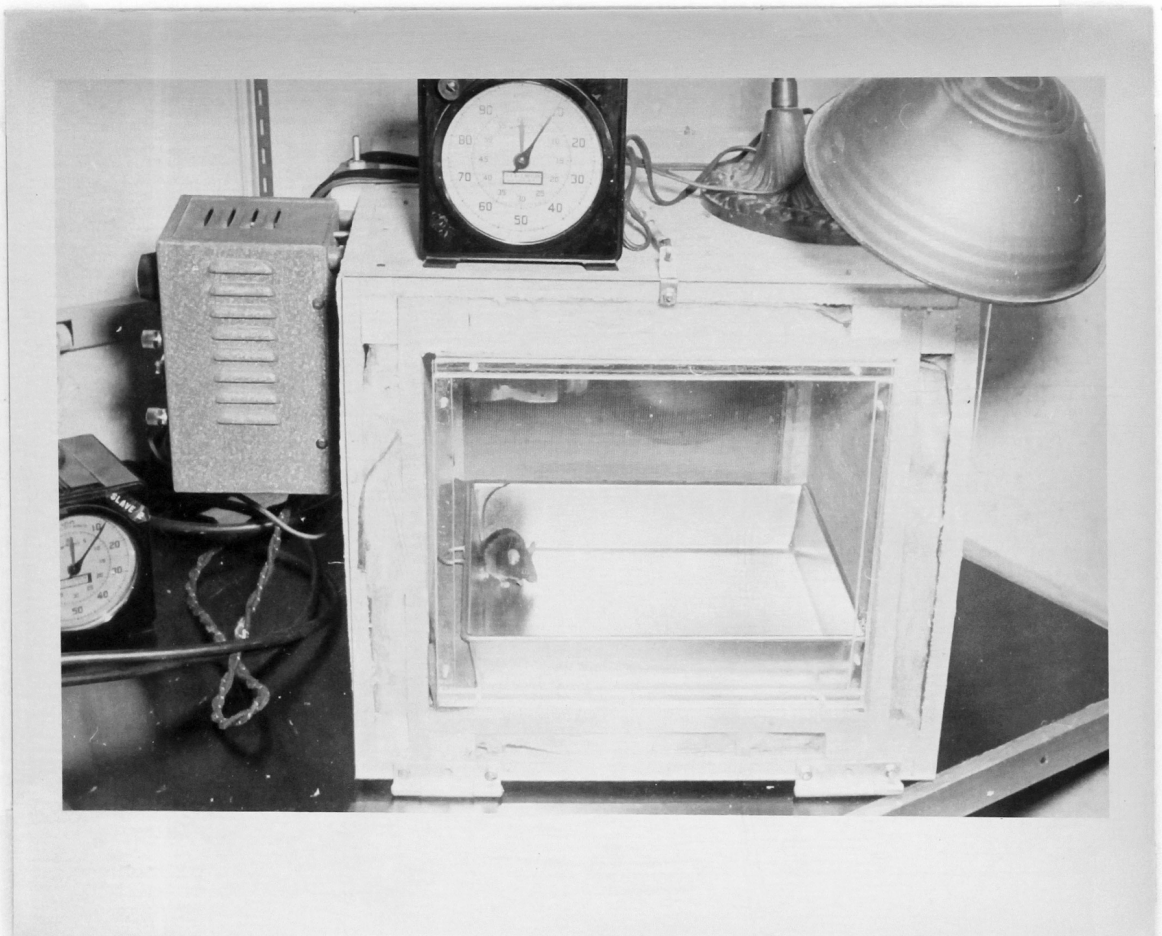

Fig. 2. Apparatus used for sound stimulation.

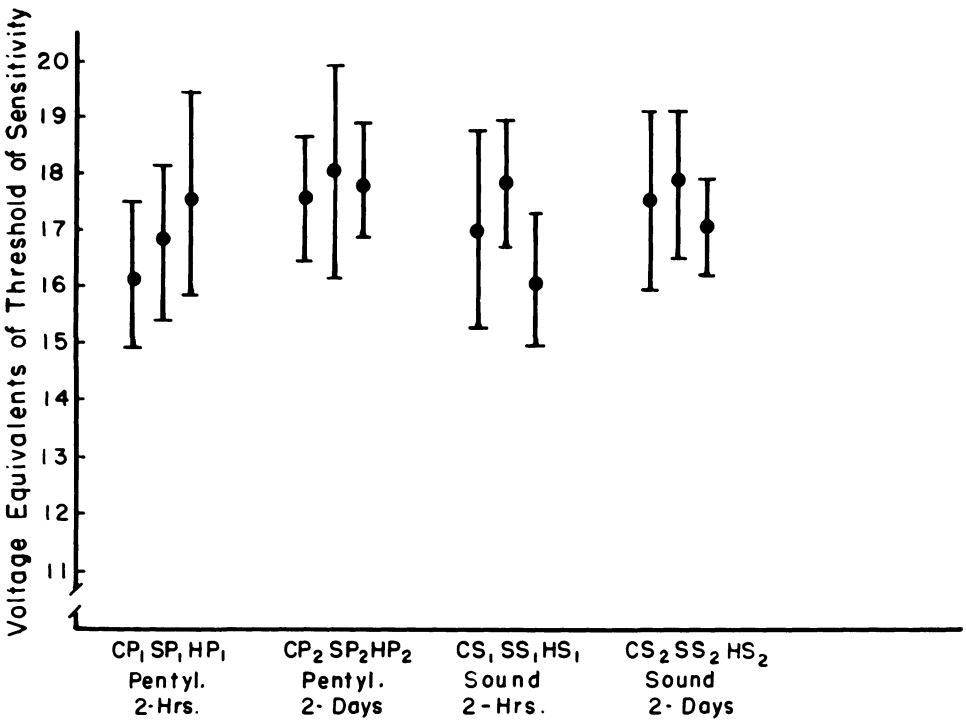

Fig. 3 Post-test grid shock threshold mean scores with 95% confidence intervals for subgroups of each agent used.

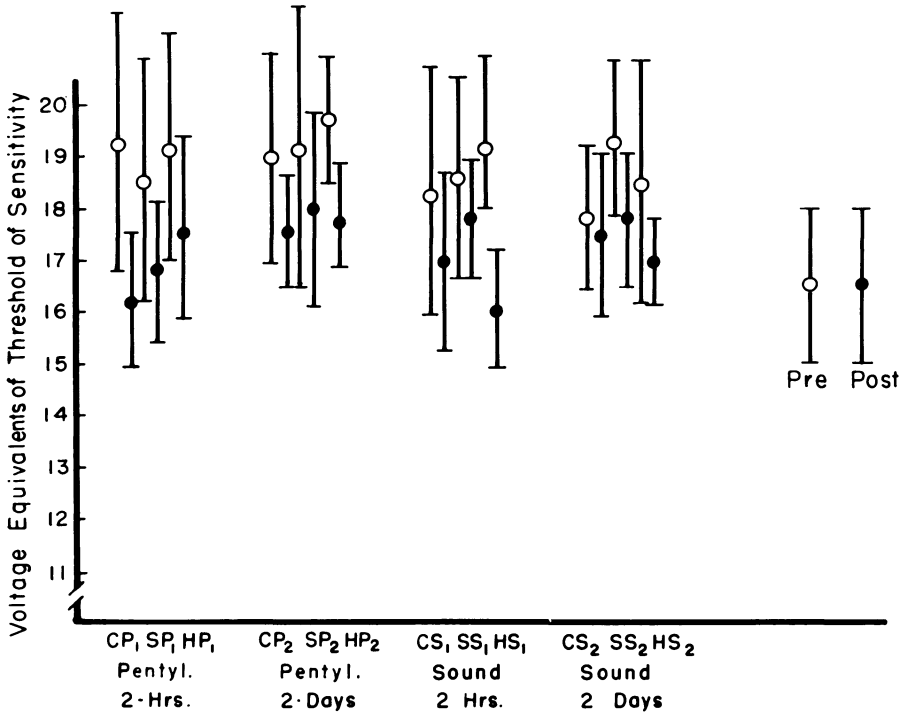

Fig. 4 Pre- and post-test mean grid shock threshold with 95% confidence intervals for subgroups of each agent used.

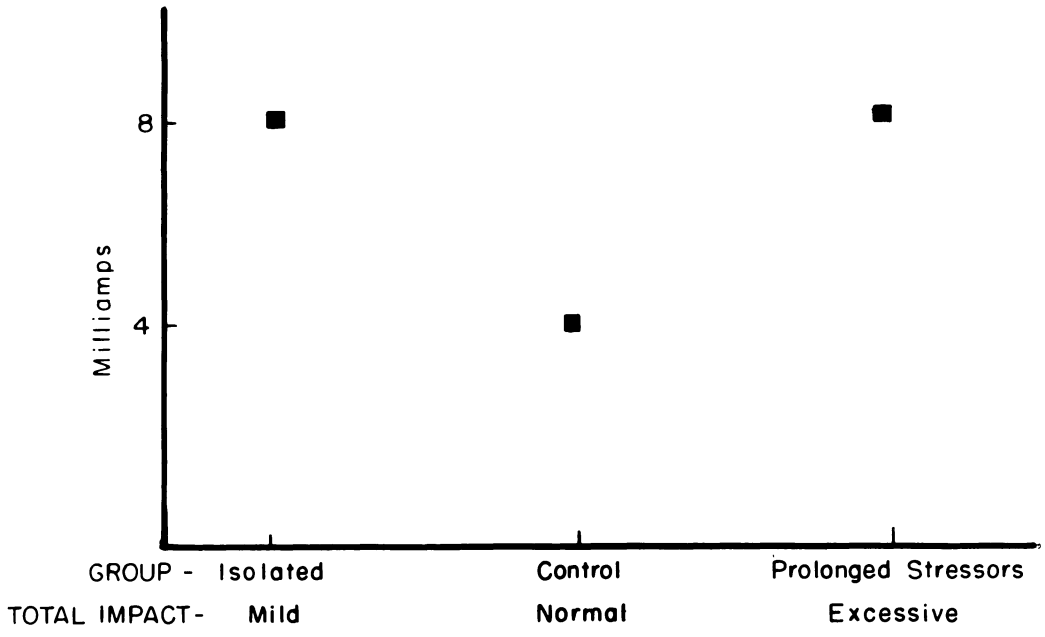

Fig.5 Griffiths' (1960) data applied to the U-shaped relationship model (Fisk & Maddi, 1961). Three groups in Griffiths' study are fitted to three levels of "total impact". See text.