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Genotype as a Determinant of Electrical and Behavioral Reactions to Shock Stimulation in the Mouse

by

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Genotype as a Determinant of Electrical and Behavioral Reactions to Shock Stimulation in the Mouse[1]

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A recent pilot study of lever-press avoidance conditioning employing a 30-second self-terminating UCS revealed large strain differences in the frequency of escape as well as avoidance behavior in mice. Although two strains (the DBA and the C57) performed the escape response equally well during the first few conditioning trials, the course of conditioning was subsequently different for the C57. Even after five training sessions and an average of 95 trials per subject, C57s allowed shock to terminate itself on over 90% of their trials whereas DBAs failed to escape the shock on fewer than 5% of their trials.[2] Behavior observations suggested that the C57 had acquired a freezing response which precluded the performance of escape and avoidance behavior. These findings appear to be consistent with the results of another study which found the DBA to be moderately efficient in active avoidance learning whereas over half of the C57s failed to meet the learning criterion even after 700 conditioning trials (Royce and Covington, 1960).

Since both strains performed the escape response equally well during the first few training trials, it is possible that the inability of the C57 to utilize reinforcement derived from shock termination may have in turn caused freezing behavior, and not conversely.. Consequently the present experiment was undertaken in order to directly assess genetic differences in freezing behavior when shock was inescapable for both strains. Since the experi-

mental procedure was adapted from Campbell and Teghtsoonian (1958), a further purpose of the experiment was to extend their findings regarding the functions relating electrical impedance to shock intensity in rats.

Method

Subjects and Apparatus The subjects were 11 naive male mice drawn from each of two highly inbred strains (the DBA/8 and C57BL/1). The apparatus was an all-metal rectangular activity cage (3 1/2 x 11 x 6") which had a floor consisting of two identical but functionally independent, spring-suspended grids which could be electrified alternately or simultaneously. A highly sensitive contact arrangement on each grid enabled the recording of the number of times the animal moved from one grid to the other plus the number of times he lurched vigorously enough to activate the grid on which he was standing. The lid of the activity cage contained two 7 1/2 watt, 110 volt frosted lights, and the entire apparatus was securely mounted in a sound proofed, air ventilated chamber which contained an observation window. The shock circuit (figure 1) consisted of a d.c. constant voltage power supply in series with a total resistance of 157 Kilohms and a Foringer grid scrambler modified to produce d.c. pulses of about 7 ms in duration. The impedance of the mouse was measured by recording on a Sanborn recorder the signal developed across a 47K resistor in series with the subject. With this arrangement it was possible to record the animal's impedance during the entire time he was being shocked.

Procedure The first step in the experimental procedure was the determination of aversion thresholds for each inbred strain. Subjects received each of five shock levels (15,20,25,30, and 35

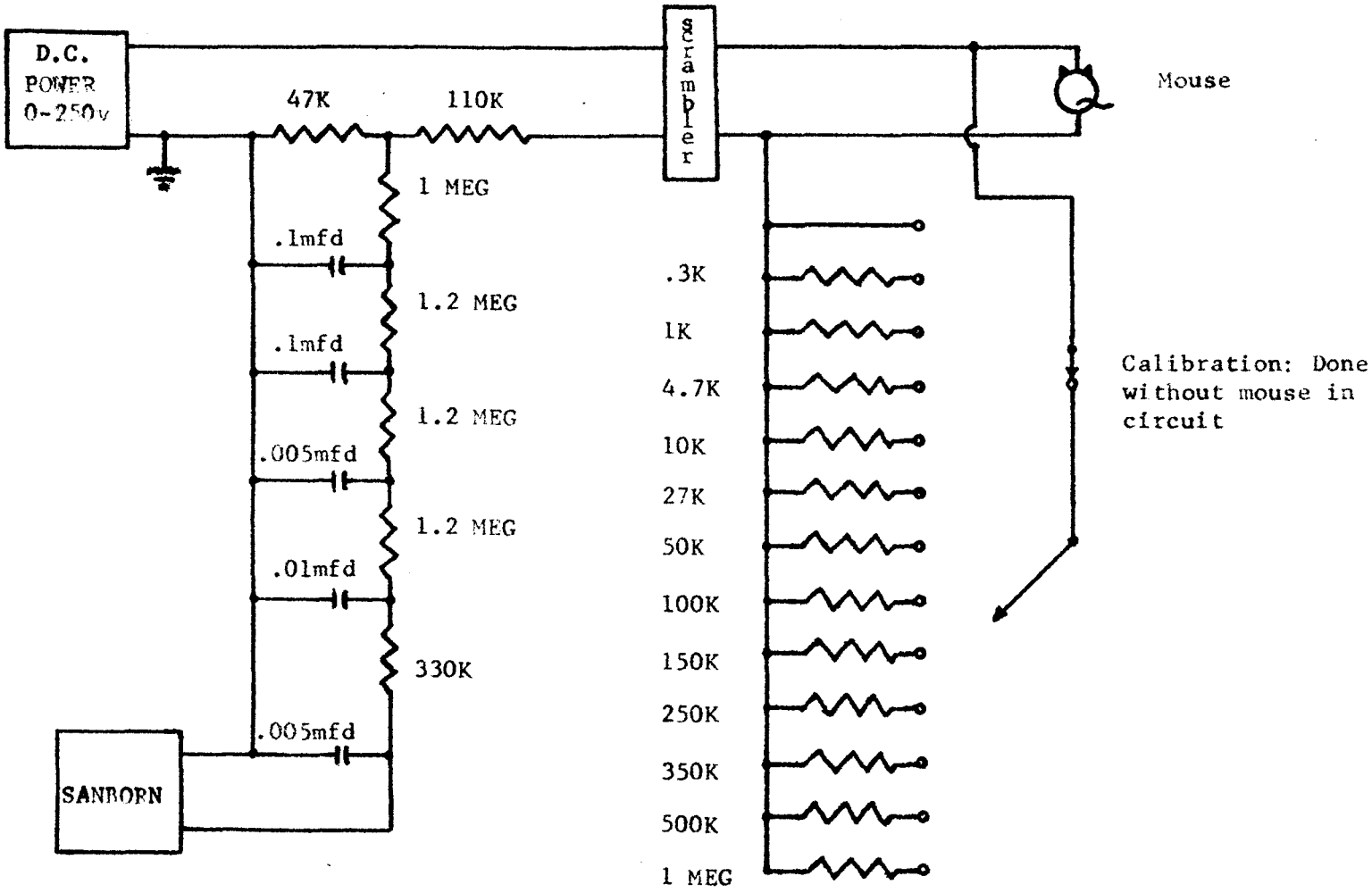

Figure 1 : "Matched Impedance" D.C. Shock Circuit

volts) in either ascending or descending order. All subjects were tested at each level for 10 minutes in a single experimental session which began with a ten minute adaptation period. The shock was alternated between the two grids and the time spent on the safe grid was recorded. The "aversion threshold" was defined as that level of shock which was avoided 75% of the time. Electrical impedance was also recorded during the 15 volt trials.

The second step in the experimental procedure was to study activity and electrical impedance as a function of shock intensity when the stimulus was applied to both grids simultaneously. Following Campbell and Teghtsoonian (1958), a decibel scale of shock intensity was constructed using the aversion threshold as the anchor point. Starting fourteen days after their threshold session, subjects received one of six levels of shock intensity each day for ten minutes. Shocks were administered in consecutive daily sessions in the same ascending or descending order assigned during threshold determination. Activity was automatically recorded every two minutes and impedance was measured throughout the trial. Continuous measurements of activity were also automatically recorded in the margin of the impedance record.

Results and Discussion

Aversion Thresholds In both strains subjects which received an ascending series of voltages exhibted higher thresholds than descending subjects, but for practical reasons the data for both orders have been combined and are presented in Figure 2. Estimates of the aversion thresholds derived from least squares regression lines fitted to these data did not differ significantly between the strains. Consequently it was decided to adopt 25 volts as the aversion thres-

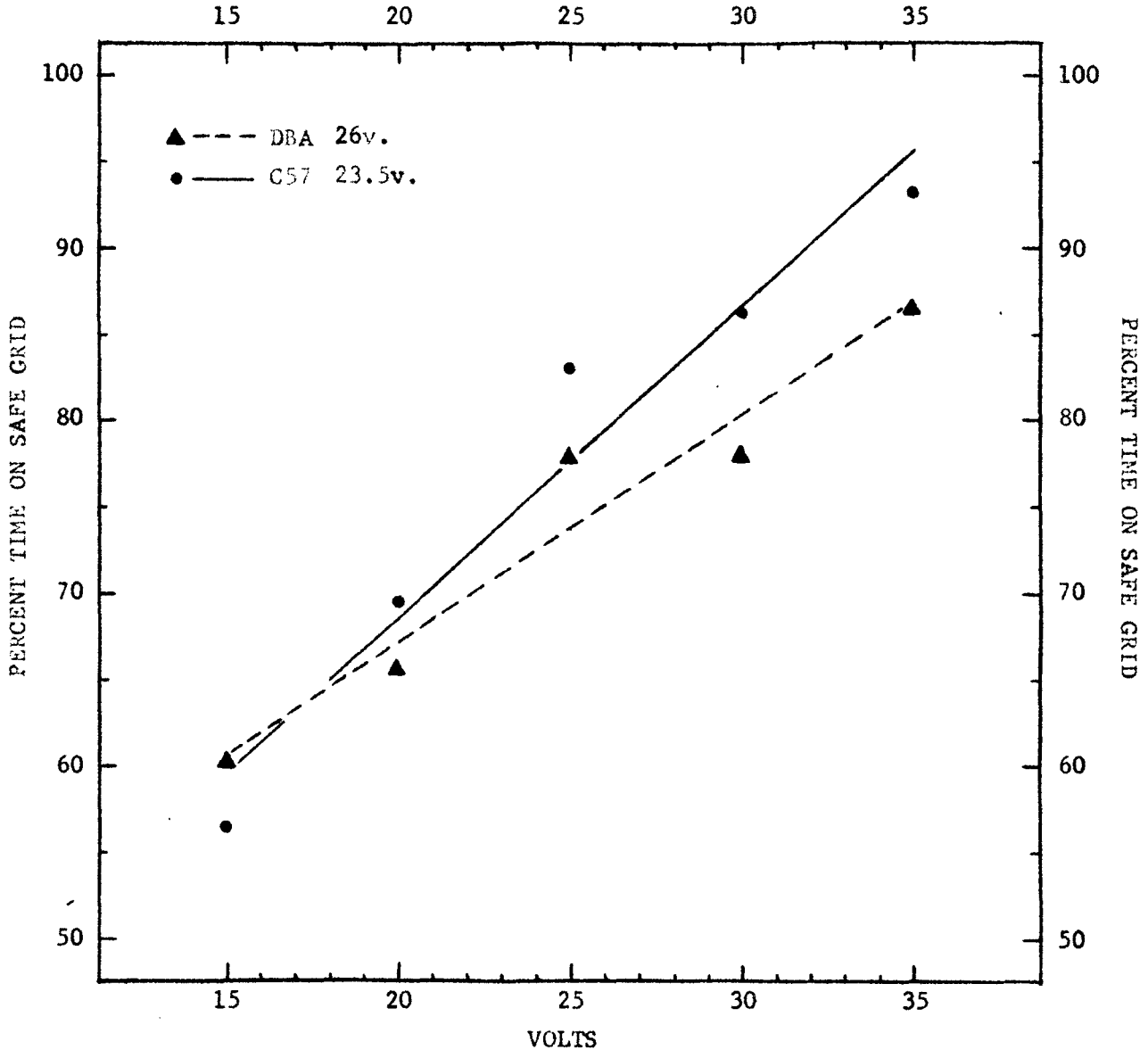

Figure 2: Aversion thresholds as a function of genotype.

hold common to both genotypes.

If the aversion threshold may be considered one behavioral indicant of subjective shock intensity in the mouse, the failure to find a significant difference on this measure clearly suggests that the strain difference in escape behavior reported earlier was not due to genetically influenced variation in the subjective intensity of electrical stimulation. Other data in the study supported the same conclusion.

Activity One purpose of the present study was to assess genetic differences in freezing behavior when shock was inescapable for both strains. "Freezing" was defined qualitatively as observed crouching on the grid bars and quantitatively as the low level of activity recorded because the animal maintained a freezing posture on the grid for varying lengths of time. The two measures were necessarily highly correlated, and it is the quantitative activity data which will be analyzed here.

Activity declined sharply after the onset of shock, reaching a stable, low asymptote before two minutes had passed. In other words, the temporal pattern of activity in both strains was one of motor excitation at the onset of shock followed by the emergence of freezing behavior within two minutes. As Figure 3 reveals, the relationship between activity and shock intensity was different for these two qualitatively different types of behavior. Here it can be seen that the activity:shock-intensity function was monotonic for the first 20 seconds of the trial where motor excitation was the predominant response, whereas it was approximately U-shaped throughout the last eight minutes of the trial where freezing behavior was observed. Analysis of the activity

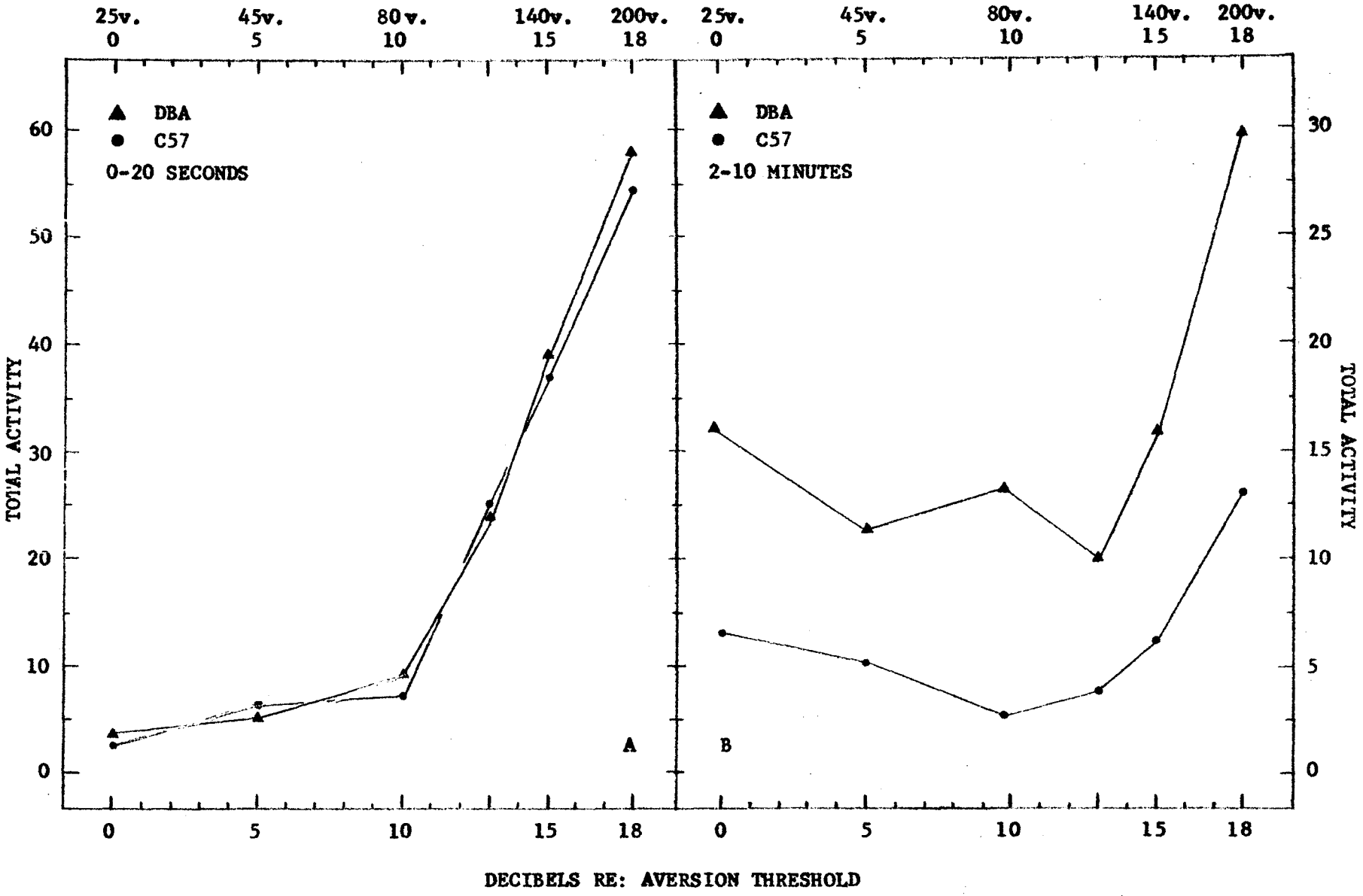

Figure 3: Activity as a function of shock intensity for different periods of the trial. Part A: Activity as a function of shock intensity during the first twenty seconds of the trial; Part B: The activity: shock-intensity relationship during each two minutes of the last eight minutes of the trial.

data at different periods during the last eight minutes of the trial revealed what Part B of Figure 3 implies, namely, that strain differences in activity were manifested throughout this section of the ttial. Figure 3 therefore suggests that the C57 and DBA differ not in their initial level of motor excitation, but in their subsequent expression of freezing behavior. Both the direction of this strain difference and its interaction with a temporal parameter agree with the earlier finding that a similar strain difference in escape performance in avoidance conditioning is observed only after a few training trials.

The activity differences observed during the last eight minutes of the trial cannot be accounted for in terms of genetic differences in susceptibility to fatigue, activity levels in non-shock environments, or initial level of subjective shock intensity. A fatigue explanation is not tenable for three reasons. First, both the vigor and duration of excitatory behavior increased with shock intensity. A fatigue theory would require a negative correlation between these two variables. Second, fatigue effects cannot reasonably explain the strain differences in activity observed at low shock intensities where the animals were not overly excited by relatively weak electical stimuli. Third, the single instance where the C57 and DBA were equally active throughout the trial occurred for descending subjects on their first trial where shock was very intense and freezing behavior had presumably not yet been acquired. Apparently the two genotypes can be equally active under the appropriate conditions. The observed strain difference in activity cannot be explained in terms of non-shock activity levels either, since the C57 is consistently more active than the DBA in non-shock environments

(cf. Lindzey, Lykken, and Winston, 1960; Thompson, 1953). Finally, the failure to find significant strain differences in aversion thresholds and in activity immediately after the onset of shock (two behavioral indices of subjective shock intensity) suggests that the electrical stimulus was equally intense for both genotypes, at least at the onset of shock. The subsequent emergence of differences in activity, however, may reflect differences in subjective shock intensity due to the activation of genetically influenced neural mechanisms which attenuate afferent stimulus input. An alternative but not necessarily substitutive explanation is that the observed difference in freezing behavior may be caused by genetic differences in neural mechanisms which inhibit reflexive motor activity. In short, strain differences in freezing behavior may be due to neural inhibition in either the afferent or efferent side of the CNS, or perhaps both (cf. Lykken, 1962; McCleary, 1961).[3]

Another result of the present study is that the observed relationship between activity and shock intensity is in general agreement with Campbell and Teghtsoonian's finding that activity is approximately a power function of shock intensity measured in decibels. This is illustrated in Part A of Figure 3, which would seem to be most comparable to their graphs since they used naive animals at each intensity level, thereby minimizing any distortion due to the acquisition of freezing behavior.

Electrical Impedance A further aim of the present investigation was to supplement Campbell and Teghtsoonian's findings regarding the relationship between impedance and shock intensity by providing similar data for mice. The relationship of the animal's electrical impedance to shock intensity observed in the present

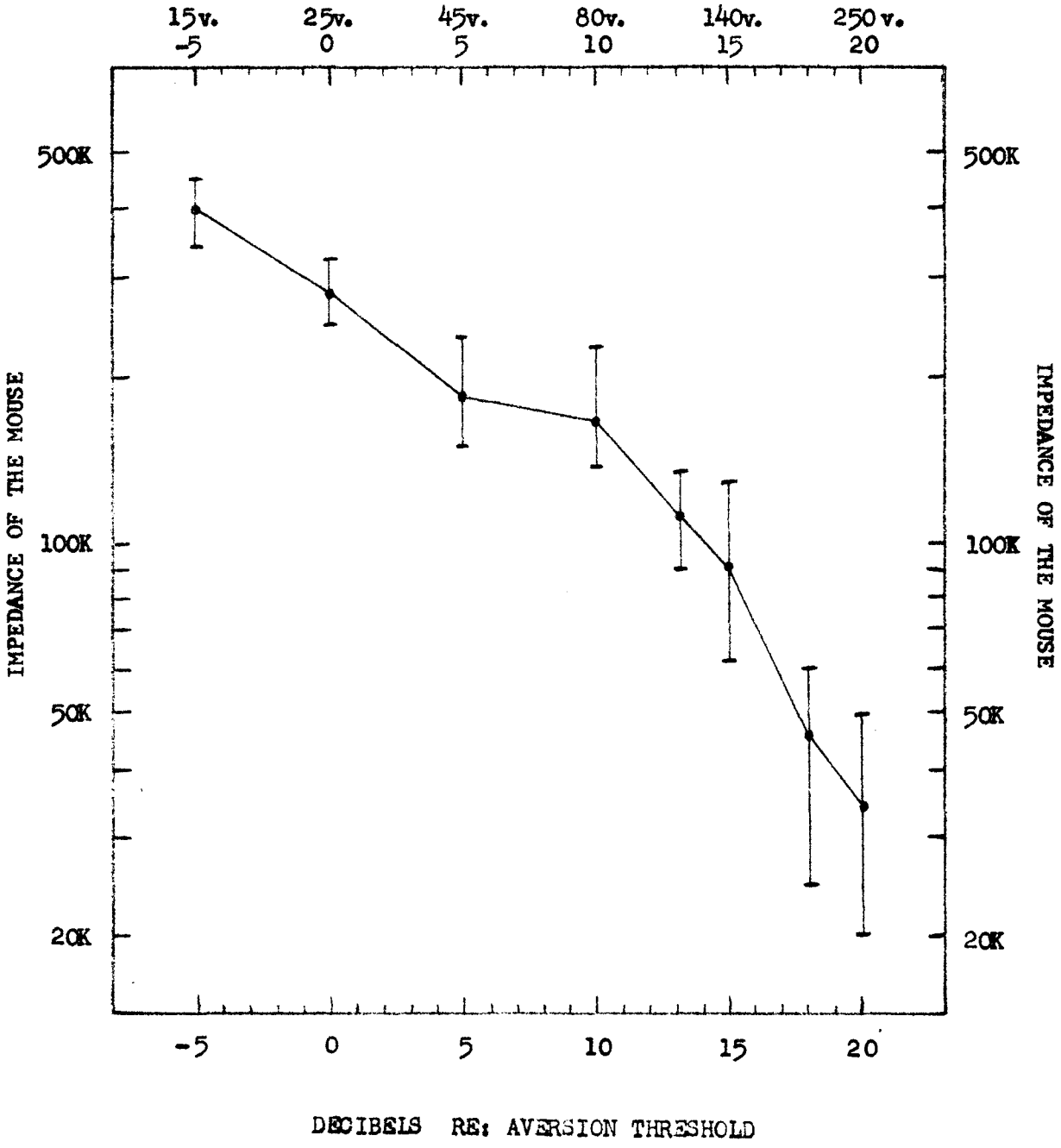

Figure 4:

Impedance of the mouse as a function of shock intensity, strains and orders combined

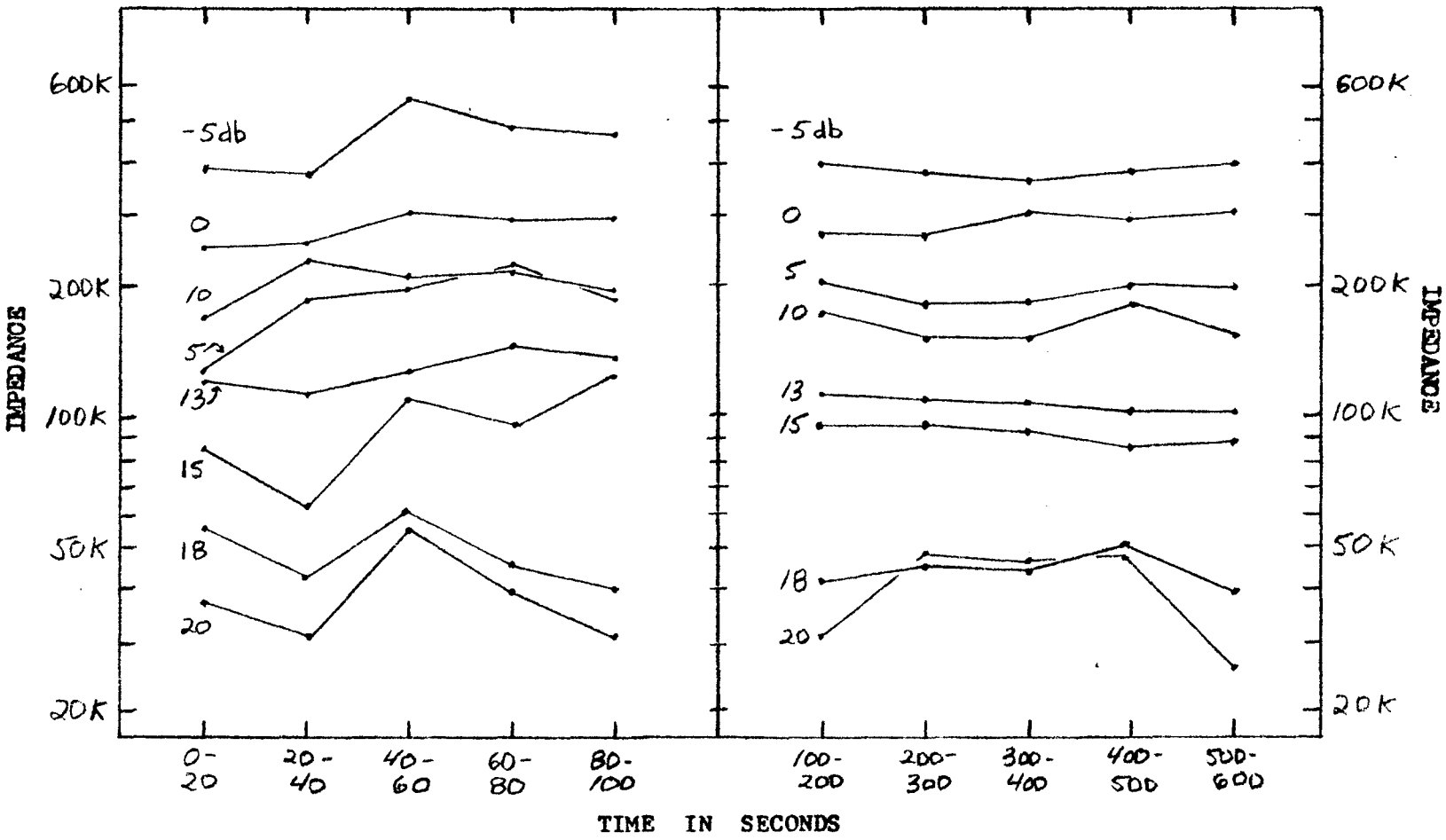

Figure 5:
Impedance as a function of time at the various intensity levels (Strains and orders combined)

study is shown in Figure 4. Since no systematic differences between orders or strains were noted, all groups were combined for impedance analyses. These data represent the average of individual medians and within-trial 20-80 percentile ranges for both genotypes at each intensity level.[4] Although the impedances tend to be slightly higher, the form of this relationship agrees with that found by Campbell and Teghtsoonian for rats. It is encouraging to note, therefore, that despite several notable variations in experimental procedure and the use of a different species, the functions relating activity and impedance to shock intensity observed in the present study generally approximate those provided by Campbell and Teghtsoonian.

Another picture of the within-trial variability in the mouse's impedance may be obtained from Figure 5 which shows the animal's impedance at different shock intensities as a function of the time it was being shocked. These data represent average individual medians during the time intervals indicated on the abscissa. The apparent stability of electrical impedance throughout each trial agrees with the findings of Campbell and Teghtsoonian. In addition to Figure 5, inspection of the impedance records suggested that impedance changed almost instantaneously when shock was applied. This finding suggests that changes in impedance due to changes in shock intensity are primarily a result of polarization and other physiological effects of the applied stimulus and not to tissue destruction itself. That tissue destruction is not a likely possibility is also indicated by the fact that the impedance:shock-intensity relationship for subjects who received a descending series of shock intensities did not differ from those receiving an ascending series. If tissue destruction were a significant factor in the

impedance drop, the damage would have to have been repaired in 24 hours in order to have obtained this result.

Summary and Conclusions

The major purposes of this experiment were to (1) study freezing behavior in two mouse strains known to differ widely in active avoidance learning, and (2) supplement the findings of Campbell and Teghtsoonian (1958) regarding the functions relating activity and impedance to shock intensity in rats.

Aversion thresholds were determined for each strain and a decibel scale of shock intensity was constructed. Using ten minute trials at each voltage level, activity and impedance were then studied as a function of shock intensity.

The major findings were (1) the strain known to be deficient in avoidance and escape behavior exhibited a significantly greater amount of freezing behavior as well, and (2) the nature of the functions relating activity and electrical impedance to shock intensity for mice are in general agreement with those for rats.

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Footnotes

1. This research was supported in part by a fellowship (number MH-20-830-D1) from the National Institutes of Health. A more complete description of the experiment may be obtained on request. The author is much indebted to David T. Lykken for both his helpful advice and use of his research facilities.
2. The F_1 hybrid cross between the C57 and DBA was also run and did not differ from the DBA parental line. Further experiments concerning this finding are in progress.
3. As conceptualized here, modulation of afferent neural activity determines subjective shock intensity.
4. Impedance measurements due to known circuit artifacts or to feces shorting out the grid were discarded from the analysis.