Shock-Induced Activity Changes, Adrenal Lipid Depletion and Brain Weight in Mice: A Genetic Study

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Abstract:
Motor activity was evaluated before and after a brief electric shock. Whereas small genetic differences in activity were apparent prior to shock, large group differences occurred following shock. C57 mice did not freeze following a shock; DBA mice showed prolonged freezing; and the F_1 hybrids froze only briefly. Backcross groups revealed segregation for postshock activity. Adrenal lipid depletion, as revealed by the absence of Sudan staining in the adrenal cortex, was detected in many male mice, but no correlation with either pre- or postshock activity was detected. Inheritance of adrenal lipid depletion clearly was not monogenetic.
Keywords: Inbred mice, Genetics, Adrenal lipid depletion, Activity, Brain weight, Stress

Article:
There is abundant evidence that motor activity is an important influence on avoidance learning rates in rodents [5,9]. The effects of many manipulations such as drugs [2,6,7,10] and lesions [1,14] appear to be mediated at least partly by nonassociative changes in activity or reactivity instead of variations in memory or associative learning ability. Genetic studies also have revealed the importance of nonassociative activity variations for strain differences in avoidance learning [6, 15, 17]. Most of this research has suggested that any treatment which increases immobility will retard avoidance learning; likewise, a manipulation which disrupts the freezing or crouching response will facilitate the performance of active avoidance responses.

It is important to understand that in a situation where a stress such as electric shock is present, changes in the behaviour of an animal fall into 2 broad categories. Associative changes result from previous experience in the situation whereby the animal learns that a painful event is correlated in space and time with a relatively innocuous event, and accordingly it modifies its response to the innocuous event in anticipation of the stress. Depending on the specific paradigm and amount of experience, the animal may become immobile, jump about wildly or execute a skillful avoidance response. However, stress by itself elicits changes in behaviour which are not learned during training and which have nothing to do with pairing of shock with another stimulus. These kinds of changes are said to be nonassociative. They are most conspicuous at a definite time following stress. For example, immobility or freezing by rats occurs as an unlearned response to electric shock [8, 11, 20]. In the avoidance learning situation, of course, nonassociative processes intermingle with associative learning. Only when opportunities for associative learning are restricted, e.g. when one stress is presented unpaired, can the unlearned stress response itself be observed clearly.

Our present interest in these problems arose from the observations that inbred mouse strains learned active avoidance at vastly different rates [24] and that the time course of corticosterone release from the adrenal cortex was also dissimilar for the same strains [18] . Subsequent research indicated that one of the strains (DBA/2) lacked the usually abundant stores of lipids which are mobilized for conversion to corticosterone in the adrenal cortex as part of the more global stress response [13]. Since the adrenal lipid depletion was shown to be a recessive character controlled by a single gene *(ald)* in other strains [16], these findings raised the possibility that genetic differences in avoidance learning might be produced in large part by different stress reactions resulting from foot shock.

For these reasons we 'undertook a study of the inheritance of shock-induced activity changes and adrenal lipid staining in mice. The correlation between these 2 phenotypes was investigated in F_1 and backcross generations. Activity was measured only after a single shock, because repeated testing would have confounded associative and nonassociative effects. Unlike several previous studies, female mice were included in the experiment; they were of special interest because they do not show the lipid depletion even in the DBA/2 strain. Body and brain weights were also observed for most mice. Brain weight was of interest because of its possible correlation with motor activity levels [17].

TABLE I

MEAN SCORES ON SEVERAL MEASURES FOR MALE AND FEMALE MICE OF 8 CROSSES

	Sex	C57* X C57	DBA X DBA	C57 X DBA	DBA X C57	DBA X F_1	F_1 X DBA	F_1 X C57	F_1 X F_1	Mode† of Inheritance
Number of	♀	12	20	24	16	11	46	16	37	
Animals	♂	22	20	18	13	7	34	18	48	
Body	♀	20.0	18.4	21.2	21.9	21.1	21.6	—	—	OVER
Weight (g)	♂	25.9	22.6	27.3	28.4	26.1	28.6	—	—	OVER
Brain	♀	0.442	0.372	0.450	0.440	0.422	0.428	—	—	DOM
Weight (g)	♂	0.454	0.370	0.438	0.444	0.420	0.416	—	—	DOM
Adrenal	♀	1.91	1.53	2.13	2.03	1.69	1.70	2.20	2.20	DOM
Cortex‡	♂	1.30	1.12	1.44	1.30	1.14	1.26	1.46	1.36	DOM
Lipid/Cortex	♀	0.633	0.416	0.528	0.522	0.282	0.422	0.578	0.539	INT
Ratio	♂	0.373	0.037	0.087	0.276	0.004	0.011	0.519	0.375	INT
Adrenal	♀	0.578	0.713	0.695	0.649	0.880	0.922	0.733	0.934	INT
Medula‡	♂	0.514	0.588	0.770	0.672	0.660	0.694	0.732	0.787	OVER

*Strain of female parent is given in the top row, and strain of male parent appears in the bottom row.
†OVER indicates overdominance, where F_1 significantly exceeds the highest scoring inbred parent; DOM means dominance or significant F_1 deviation from the mean of the inbred parent scores but without overdominance; INT denotes intermediate inheritance or insignificant deviation from the midparent value.
‡Derived from mean area (mm^2) of structure in largest sections of right and left adrenals for each mouse.

METHOD

Animals

Parent mice obtained from the Jackson Laboratory, Bar Harbor, Maine, included the inbred strains C57BL/6J and DBA/2J and their F_1 hybrid B6D2F_1 /J. These adult mice were then mated in several combinations to produce the number of offspring indicated in Table 1. Offspring were weaned at 20 days of age and were housed with littermates of like sex with the free access to water and dry lab chow.

Apparatus

Activity was measured in a black Plexiglas box 15 cm × 15 cm × 25 cm high with a grid floor of 0.16 cm bars 0.95 cm apart. Locomotion was detected by the breaking of a beam of infrared light from a light-emitting diode (GE SSL-35) to a photocell-amplifier (2N5780). Three parallel light beams 5.2 cm apart and 1 cm from the grid were oriented in one horizontal direction, while a second set of 3 beams was similarly oriented orthogonal to the other 3. Photocells in one orientation were connected to a logic network such that a pulse from one cell would be delivered to the counter only if 1 of the other 2 beams in that direction had previously been broken; thus, counts were delivered for gross locomotion about the box but not for repeated, small movements in front of a single photocell. The two sets of three photocells operated independently, but the 2 outputs of their respective logic circuits both activated the same counter via an OR gate.

Output of the photocell's logic circuits went to a programmable counter (Hewlett-Packard, 5326B) whose output in turn went to a printer (H-P, 5055A).

Shock was delivered at 180 µA, constant current, from a 400v AC supply via the grid floor using a diode bridge scrambler. A 10K ohm resistor in series with the mouse was connected via a full-wave rectifier to an amplifier and thence to a Schmitt trigger such that a logic-level pulse was delivered to a counter whenever the current through the mouse exceeded 100 µA. If a mouse was receiving the full shock, a maximum of 120 counts per sec would be recorded (since 60 Hz AC shock was used); when the mouse jumped or ran about, fewer counts would

result. Of course mice receiving the same number of pulses would not necessarily receive the same total amount of shock, because a threshold trigger device was used, but at least they would receive much more comparable amounts of shock than 2 mice simply equated for the time the shock is available An analog record of current flowing through the mouse was made with a Brush 220 oscillograph.

All testing was done under dim red light (2.2 loot candles) during the dark phase of the normal day—night cycle.

Procedure

Half of the mice in each group received a single foot shock, while the other half were used as nonshocked controls. Control mice were simply placed into the box for 15 min, and their locomotor activity count was printed out every 10 sec. Experimental mice were placed into the box, and 5 min later they received a single foot shock; activity was then recorded for a further 10 min. Activity, was printed out every 10 sec, The shock was of 60 pulses duration, regardless of how long it required the mouse to receive that number of pulses; it corresponded to 0.5 sec. of actual shock going through the mouse. This method d shock control was necessary to compensate for the different amounts of jumping during shock by these toe' strains [25].

All mice were from 70 to 80 days of age at the time of activity testing.

Histology

Fen days following testing, each mouse was perfused intracardially with saline followed by 10% Formalin-saline. Left and right adrenal glands were removed and placed separately into 10% Formalin. Brains were also removed and stored in formalin. Prior to weighing to the nearest mg, parts of the brain which often were destroyed during extraction were carefully trimmed away for all brains; these included olfactory bulbs, optic nerve, trigeminal nerve, paraflocculi and spinal cord. This procedure resulted in reduced within-group variability.

Each adrenal gland was subsequently sectioned at 33 µm on a freezing microtome, and then sections were stained with Sudan Black B for lipids in the adrenal cortex. Tracings of the sagittal section of greatest area of each adrenal were made with a projecting microscope to show the various zones. Areas of the various zones and the lipid-rich region were measured with a planimeter. Then the ratio of the area of lipid staining to total area of the adrenal cortex was calculated, and the scores for the left and right adrenal glands were averaged to give a score for each mouse.

RESULTS AND DISCUSSION

Behavioral testing was done for all but the $F_1 \times C57$ and $F_1 \times F_1$ crosses; these groups were studied at a later time to clarify the inheritance of adrenal lipid staining.

Statistical analysis of behavioral data, body weight and brain weight was done using an unweighted means analysis of variance with a Genotype by Sex design. Among all variables analyzed, only body weight showed a significant sex difference, while no significant interactions with sex were detected. For this reason sexes were pooled in subsequent presentation of results and in the figures. Comparisons among the various genotypic groups were performed using planned contrasts (orthogonal comparisons using F test). Because the reciprocal F_1 groups did not differ significantly on any variable, their data were considered together in subsequent presentation; the same outcome held for reciprocal backcrosses, and their data were also pooled for presentation. Reciprocal F_1 and backcross groups were always separated for analysis of variance, however.

Activity (counts per sec) is portrayed over 30 sec blocks the C57 and DBA inbred strains and the pooled F_1 hybrids in Fig. 1.

Prior to shock, group differences were small but significant $F(5,110) = 3.1$, $p = 0.01$). All groups exhibited a steady but moderate decline in activity over the first 5 min. A similar pattern of results occurred for control

animals.

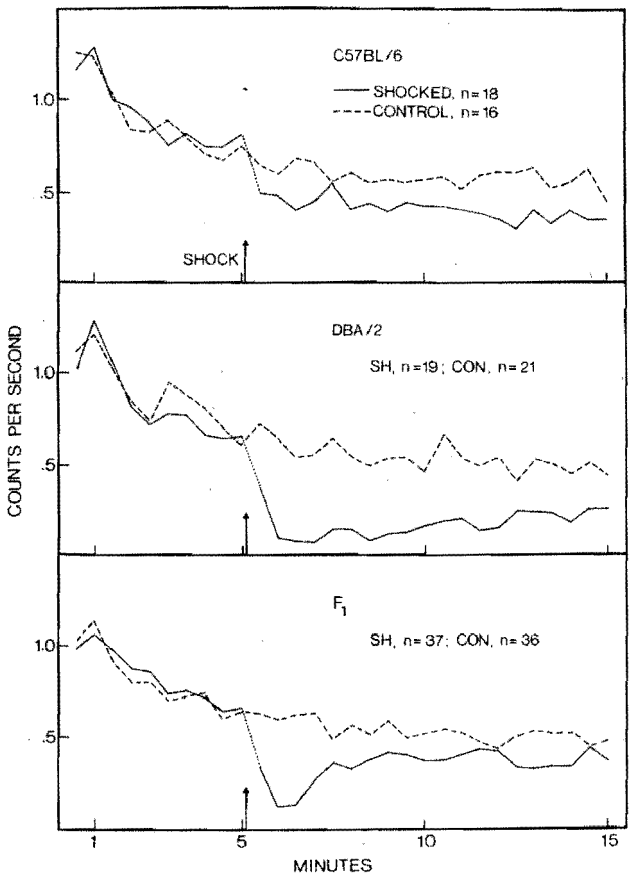

FIG. 1. Motor activity (photocell beam crossings per second) for 2 inbred strains and their reciprocal F_1 hybrids (pooled). Shocked mice (solid line) received 0.5 sec of electric shock after 5 min in the grid box (arrow), while control mice (broken line) received no stimulation. Each group represents an approximately equal number of male and female mice.

Reaction to the shock itself varied widely and significantly; as indicated by the time required for the animal to receive 0.5 sec of actual shock. C57 × C57 mice took much less time (1.73 sec) to accumulate 0.5 sec of shock than did DBA × DBA mice (3.55 sec; $p < 0.001$). Previous research in this laboratory has shown that this is so because DBA/2J mice jump more often and higher than do C57BL/6J mice during electric shock [25] ; inspection of the oscillograph recordings from the present experiment was also consistent with this explanation, because DBA mice had many long intervals without shock. Times required by the F_1 hybrids were very close to the mean of the inbred parent strains (2.54 sec), while the backcrosses to DBA were close to the values for the DBA strain. The presence of such large differences in reaction to shock emphasizes the importance of equating the amount of shock actually received.

Following shock, activity declined by a small amount for the C57 strain, as judged by comparison with nonshocked controls (Fig. 1), but activity never became really low. DBA mice, on the other hand, showed a dramatic decline in activity within 1 min after shock, and their activity levels showed only slight recovery towards control levels after 10 min. F_1 mice exhibited a very interesting, intermediate pattern (Fig. 1); they showed a substantial decline in activity 1 min after shock, but they recovered to nearly control levels within 2 to 3 minutes. Thus, hybrids revealed aspects of shock-induced activity changes which were characteristic of both parent strains. The mean activity of F_1 mice over the 10 min following shock did not differ significantly from the mid-parent value. Behavior in the backcross groups was highly variable; some subjects resembled the C57 strain, some behaved like the DBA strain, and others were more like F_1 mice.

Since much of the decline in activity appeared to be the results of freezing or immobility following shock, a

measure of freezing was derived for each mouse using a strict criterion of freezing. Only a 30 sec period wherein at most one photocell crossing occurred was regarded as a period of freezing. The number of periods of immobility in the 10 min following shock is shown for each mouse in Fig. 2. The inbred parent strains differed greatly ($p<0.01$), while F_1 hybrids showed an intermediate status. Backcross mice showed a wide range of freezing scores, many of which were more extreme than those of even the DBA parent strain. These backcross results clearly indicate segregation at loci which exert a profound genetic effect on activity following electric shock. A comparable freezing measure during the last 10 min of activity for control mice revealed no significant group differences, the overall mean score being less than I period of freezing in 10 min.

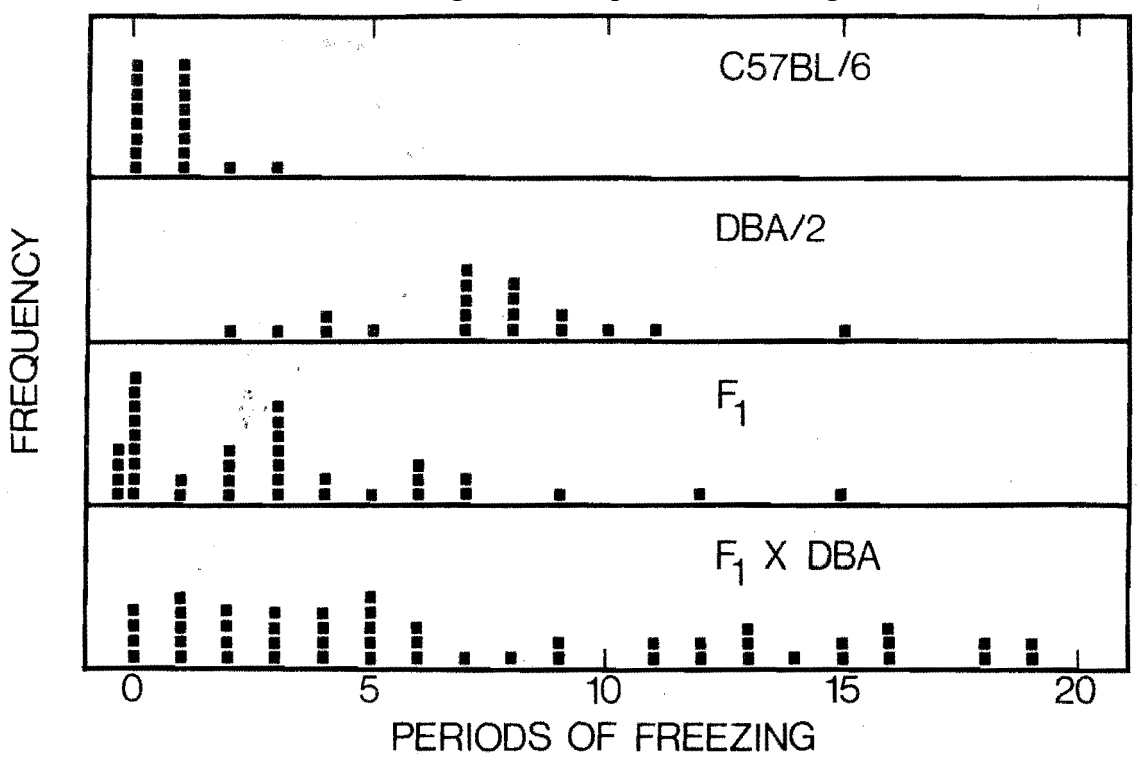

FIG. 2. Periods of freezing following 0.5 sec of electric shock for individual mice of two inbred strains, pooled reciprocal F_1 hybrids and pooled reciprocal backcrosses to DBA. A period of freezing was defined as a 30 sec period in which no more than one photocell count occurred. Hence, a maximum score of 20 was possible in the 10 min observation period following electric shock. Each point represents the number of 30 sec periods out of a possible 20 which an individual mouse spent freezing following shock.

Summarizing the results of behavioral testing, small genetic differences in activity were observed in the absence of electric shock. Following shock, however, large differences were manifest; C57 mice did not freeze at all, DBA mice showed prolonged freezing, F_1 hybrids generally froze for a short while and then resumed activity, and backcross mice showed great variability in behavior. Inheritance was probably polygenic, judging from backcross results.

Mean values for several physical variables are presented separately for females and males in Table I. Highly significant differences in body weight were found among genotypes, $F(5,231) = 6.2$, $p<0.001$, and between sexes ($p< 0.0001$). Hybrids were heavier than the heaviest inbred parent strain, indicating overdominance. Brain weight varied significantly only among genotypes, $F(5,231) = 35.4$, $p<0.0001$; no sex difference was. present. The large brain weight difference between C57 and DBA was highly significant ($p<0.0001$), and the F_1 hybrids significantly exceeded the midparent value ($p<0.01$) but were not significantly different from the C57 score, indicating complete dominance. Distributions of brain weights revealed that inheritance was polygenic, because the backcross distribution was unimodal at a value approximately intermediate to the scores of DBA and F_1 mice.

Analysis of the adrenal gland morphology was based upon data from all mice utilized in activity testing as well as additional mice from a backcross of F_1 females to C57BL/6 males and an F_2 cross of F_1 mice. Cross-sectional

area the adrenal cortex showed substantial influences of Genotype, $F(7,346) = 15.9$, $p<0.001$, and Sex , $p<0.001$); there was also a significant Genotype by Sex interaction. $F(7,346) = 2.5$, $p<0.02$. Planned comparisons revealed that, among females, C57 and DBA differed greatly ($p< 0.01$) and that F_1 mice exceeded the midparent score ($p<0.01$); significant overdominance was present ($p< 0.05$). Backcrosses to C57 and DBA also showed a large difference ($p<0.01$). Among males, the parent strains did not differ significantly, but F_1 did exceed the midparent SCCL ($p<0.05$). Backcrosses to C57 and to DBA were different ($p<0.01$). In general genetic differences were much greater among females than among males, but heterosis was present for both sexes.

Area of the adrenal medulla, measured similarly, also revealed significant effects of Genotype, Sex and Genotype by Sex interaction (all $p<0.01$). Among females, planned comparisons obtained only one significant effect: backcrosses to the 2 parent strains were different ($p<0.01$). Among males, significant heterosis ($p<0.01$) and over dominance ($p<0.05$) were present. Thus, area of adrenal medulla showed relatively moderate genetic influences.

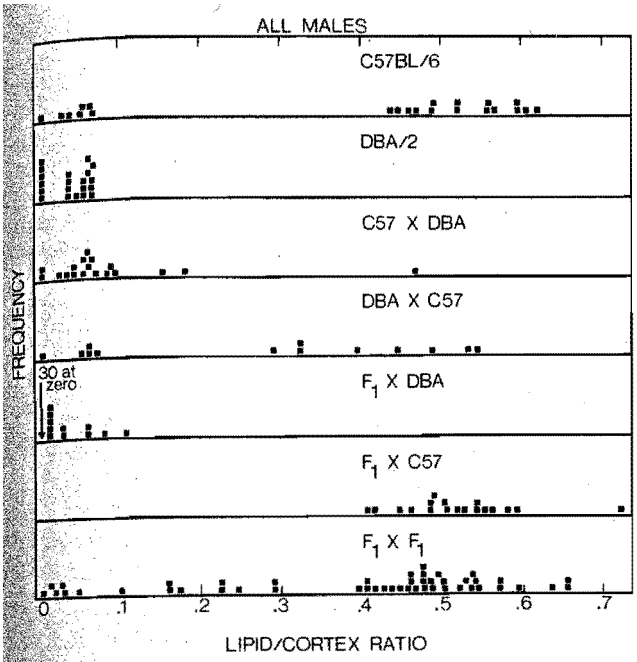

FIG. 3. Ratio of area of lipid staining in adrenal cortex as revealed by Sudan Black B staining to area of adrenal cortex itself in adrenal cross-section of greatest area for each male mouse of several genetic groups. Each score is the mean of values for left and right adrenals.

When the area of the adrenal cortex which showed clear lipid staining was compared to the total area of the cortex in the largest section, some adrenals were observed to be nearly free from lipid staining and were therefore affected by adrenal lipid depletion. Many males showed this condition, but only 2 females (in DBA × F_1) showed staining of less than 15 percent of the cortex. Because of the lack of variance in several groups of males, analysis of variance on lipid/cortex ratio was deemed inappropriate. Analysis of females revealed substantial inbred strain ($p<0.01$) and backcross ($p<0.01$) differences but no dominance. For males, the situation was somewhat complex. s portrayed in Fig. 3. As noted previously, all DBA males showed adrenal lipid depletion, but so did a few C57 and numerous F_1 males. Males in the backcross to DBA all owed depletion. Hence the inheritance of the depletion phenotype appeared to show dominance, and a substantial degree of environmental variance was also present. Since these results were at odds with previous suspicions about *ald* in DBA mice [10], the backcross to C57 and the F_2 cross were performed in order to clarify the mode of inheritance further. None of the F_1 × C57 males showed lipid depletion, which means that no segregation was present within a backcross of F_1 to either inbred parent strain! Only in F_2 was segregation obtained; 15 of 48 males showed some lipid depletion, but only six showed severe depletion like DBA males. From these results it is clear that the difference between C57 and DBA must be the manifestation of more than 1 pair of alleles. The presence of a major gene-environment interaction is also a real possibility.

Correlates of the lipid/cortex ratio could not be estimated in most groups of males owing to lack of variance. Among C57 × C57 and DBA × C57 males, where a wide spread of scores occurred, no significant correlations with either pre- or postshock behavior were found. Within groups of females, no consistent pattern of correlations emerged. No significant correlations between brain weight and activity were found in any group.

GENERAL DISCUSSION

Adrenal lipid depletion was observed frequently in this study, but it appeared to have no significant relationship with behavior. Three major kinds of evidence argued against a phenotypic correlation. (a) Only males showed adrenal lipid depletion; nonetheless, no sex differences were observed in pre- or postshock activity or postshock freezing; (b) In male mice from backcrosses to DBA, segregation for the depletion phenotype did not occur, but a wide range of postshock freezing was apparent; (c) In homogeneous genetic groups of males where a wide range of lipid staining scores existed, no correlation with behavior was present. Neither did the size of adrenal cortex, adrenal medulla or brain correlate reliably with any aspect of behavior before or after shock. Thus, all phenotypes examined in this study were influenced strongly by genetic variation, but they appeared to be inherited largely independently. It is interesting that Shire [221 found a correlation between adrenal weight and exploratory activity across 3 inbred strains but not in a segregating backcross generation.

It is very clear from this experiment that adrenal lipid depletion in DBA/2J males is not the manifestation of a single gene. Whatever the nature of genetic control may be, it is characterized by dominance for depletion, unlike the recessive action of the *ald* gene in AC strain mice [16]. Another recent report also has indicated lack of segregation for Sudan staining in a backcross to DBA/2J [13]; this result occurred when C57BL/10 was the other inbred parent strain. However, when AC and DBA/2J strains were used, the backcross to DBA showed definite segregation for Sudan staining, thereby demonstrating that *ald* in AC mice is not allelic with genes producing depletion in DBA. In addition, Doering *et al.* [13] failed to find segregation for corticosterone production capacity in the backcross to DBA/2J. The really puzzling thing about the depletion phenotype in the present study is that it failed to show segregation in the backcross to either C57BL/6J or DBA/2J; only in F_2 did segregation occur. Given the rare appearance of Sudan staining in the intermediate range of 0.1 to 0.4, some kind of a physiological switch mechanism may exist to produce lipid depletion in mice which possess a sufficient abundance of DBA-type genes. This idea is made plausible by the Observations that even DBA males show lipid staining prior to puberty and that depletion is androgen-dependent [13] . The present finding that a wide range of lipid staining may be found among males of homogeneous genetic constitution also suggests that the switch mechanism may be subject to an environmental influence.

Motor activity prior to electric shock showed minor genetic differences in this study, but large genetic influences were apparent following shock. This incongruence has important implications for attempts to correlate activity with avoidance learning. Correlations between shock avoidance learning and activity measured without shock in another situation may be low or even negative [19] , while correlations between intertrial activity during avoidance training and learning rate are positive [1517]. Whether genetic differences in learning rate are in fact influenced strongly by nonassociative changes in activity level must be investigated for each particular task, perhaps by studying animals receiving noncontingent shock or by using yoked controls. Activity after a single shock may be especially important for one-trial inhibitory avoidance learning. Unfortunately, research claiming genetic variation in one-trial learning [23] has not included pertinent controls to separate the influences of learning and activity. In more complex, active avoidance paradigms the activity levels after repeated shocks and reaction to subsequent shocks appear to be more relevant. The postshock activity measure and a repeated shock procedure have been useful in explaining certain strain-drug interactions in several multiple-trial avoidance tasks [3,4] , as well as elucidating the factors subserving the reacquisition of avoidance responses or avoidance following inescapable shock [21] The main point, however, is that much more careful work needs to be done in future genetic research in order to separate the influences of associative learning and non-associative activity changes during shock avoidance.

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