

TIMIDITY AND FEARFULNESS OF LABORATORY MICE: AN ILLUSTRATION OF PROBLEMS IN ANIMAL TEMPERAMENT*

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ABSTRACT—Two inbred strains of mice and their derived generations were tested for home cage emergence and open field behavior under two levels of environmental stimulation. The least timid (fast emerging) genotype was found to be most fearful (high defecation in open field), whereas the most timid strain was least fearful. In addition, exposure to a loud noise during testing consistently resulted in a decrease in emergence latency and an increase in open field defecation, i.e., environmental stimulation sufficient to decrease timidity increased fearfulness. This apparent paradox illustrates a major problem in interspecific behavioral comparisons: *a priori* analogic reasoning from human theory to animal model, without regard for the meaning of constructs in the behavioral organization and evolutionary adaptation of the species studied, often results in rigorous investigation of operationally defined behavioral constructs devoid of meaning.

INVESTIGATION of temperament variables in animals has been an active area of research throughout the history of experimental psychology (Lindzey, Winston, & Manosevitz, 1961). Research in this domain is usually concerned with broaching important problems of human emotionality and personality organization through controlled experimentation employing infrahuman species (Lindzey, Lykken & Winston, 1960; Deneberg, 1967; Scott, 1967). One of the best validated measures involves elimination in a brightly illuminated arena. Defecation and urination in a strange open field situation were introduced as indicants of emotionality in rats by Hall in 1934. Later, the test was more specifically claimed to be related to fearfulness (Farris & Yeakel, 1945; Hall, 1951). In various forms this test is the most widely employed measure of animal temperament. It has been demonstrated that defecation in the test situation is not directly related to basal levels of elimination (Lindzey, Winston, & Whitney, 1964), and that environmental manipulations designed to increase the fearfulness of the test, such as increased light or noise during testing (Broadhurst, 1960; McReynolds, Weir, & DeFries, 1967; Dixon & DeFries, 1968), lead to an increase in elimination.

From the time of its introduction, the open-field test has been related to the investigation of inheritance (Hall, 1938), and studies abound which demonstrate

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differences in emotional elimination among inbred strains of (i.e., Lindzey, 1951, Thompson, 1953). In addition, the mode of inheritance and heritability of this character have been investigated in both rats and mice (Broadhurst, 1960; DeFries & Hegmann, In press). A measure of the subject's ambulation in the open-field arena is usually taken in addition to the elimination score. For this measure, strain differences are also present in both rats and mice (Broadhurst, 1958; Thompson, 1956; Southwick & Clark, 1968), and sophisticated genetic studies have been performed (DeFries & Hegmann, In press).

Open-field activity is often found to be negatively correlated with elimination (Hall, 1936; Hegmann & DeFries, 1968; DeFries & Hegmann, In press). This correlation is a consideration in the construct validity of the test as an indicant of emotionality or fearfulness to the extent that lessened motor activity, as related to freezing behavior, is elicited by the stimulus conditions inducing emotional elimination (Hall, 1936; Broadhurst, 1960). But in a forced confinement situation such as the open field, a motility measure is at best an ambiguous indicant of fearfulness. Increased ambulation may be a result of low fear and prepotent exploratory tendencies, with low ambulation being related to fear-induced freezing. On the other hand, one could reason that heightened motility resulted from attempts to escape from a fearful situation, while animals with low ambulation scores found the confinement non-fearful. Escape and exploration tendencies measured by ambulation and their relation to an underlying fear state of the subject are probably nonmonotonic and confounded to an unknown extent in open field activity measures (Glickman & Hartz, 1964; Johnson, 1964; Welker, 1957).

A number of other testing situations, such as Stone's Stovepipe Test (Stone, 1932), and McClearn's Hole-in-wall (McClearn, 1959) have been developed as more readily interpretable indicants of fearfulness or timidity as manifested in ambulatory behavior. The stovepipe test consists of allowing a food-deprived animal access through a darkened runway (section of stovepipe) to a brightly illuminated goal box containing food. Latency to leave the dark pipe for the bright goal box is usually considered to be a positive indicant of timidity (Lindzey, Lykken & Winston, 1960). The hole-in-wall test utilizes a speed score as a positive measure of fear; here the subject is placed in a brightly lit compartment and can climb through a hole into a darkened area (McClearn, 1959). Genotype has been shown to be an important factor in individual differences on both of the above measures (Lindzey, Lykken, & Winston, 1960; McClearn, 1959). Although these situations appear to be less ambiguous than open field activity in their relation to fearfulness, an important dimension, that of familiarity, is not experimentally manipulated. The "secure" sections of both the above apparatus, the dark runway of the stovepipe test and the darkened compartment of the hole-in-wall, are just as unfamiliar to the subject as are the "fearful" locations and thus lessen the interpretability of the measures. Indeed, some investigators have taken latency to *enter* the dark pipe as a positive measure of timidity (Lindzey, Winston, & Manosevitz, 1963). That familiarity with the non-fearful segment of a test situation is an important consideration in tests of fearfulness or timidity has been indicated by others (Berlyne, Bindra, 1948; Glickman & Hartz, 1964; Telleger, 1963; Walker, 1959).

Tellegen (1963) designed an apparatus to measure fearfulness whereby mice were allowed access to an illuminated runway from their darkened home cages. He found large strain differences in latency to emerge from the home cage and interpreted this as being due to fear. Manosevitz (1965) used a similar situation and recorded latency to emerge from the home cage in a study of hoarding. In these investigations the familiar home cages consisted of compartments specially constructed for the purposes of the studies in which the animals lived for a short time prior to testing.

The present investigation also uses emergence from a darkened familiar home cage into an illuminated, less familiar area as an indicant of timidity. The emergence test differs from that used by Manosevitz (1965) and Tellegen (1963) primarily in two respects: First, the "home cages" were similar to the standard laboratory cages in which the subjects had lived most of their lives; and second, the area into which the animals were allowed access was very similar to an open field arena in which the subjects were also tested. Since a variable of animal temperament related to fearfulness or timidity should be affected by the amount of stress induced by the testing situation, subjects were tested under two levels of environmental stimulation. The inclusion of both well characterized genotypes and environmental stimulation facilitated an examination of the validity of the operationally defined constructs of timidity and fearfulness.

METHOD

Subjects

A total of 256 animals from the C57BL/1Bi and JK/Bi inbred strains and their F₁ and F₂ generations were used. The C57 and JK parental strains had been inbred by brother-sister matings for 59-61 and 81-84 generations respectively at the time of the study. From birth until weaning on the 24th day, single litters were maintained with their mother in 15 x 30 x 15 cm wood cages with floors covered by about 2.5 cm of wood chips. At weaning, littermates of like sex were housed together in similar cages. At 60 days of age, subjects were separated into 10 x 24 x 13 cm metal cages, and were housed individually throughout the study. The floor and front of the cages were of .6 cm hardware cloth; the sides, back, and top, were solid. At 119 ± 15 (range) days of age, the subjects were transferred to test cages identical to the individual cages above except for a removable metal insert covering the back wall. On the first day of the open field test animals were 125 ± 15 days of age; emergence testing began 11 days later.

Treatment Groups. Subjects were tested under one of two levels of environmental stimulation. Increased noise is a common stressor with high face validity (Broadhurst, 1960). For one group, a constant low level of background noise was provided by an electric fan and a small window-mounted air conditioner. The high stress treatment involved subjecting the animals to a loud and variable noise while in the test situation. The noise was from a recording of vocalizations made by a mouse receiving strong electrical stimulation, and was played at maximal volume from a *Wollensak T-1500* magnetic tape recorded. The number of subjects in each strain x sex x treatment group is given in Table 1.

TABLE 1
Number of subjects of each genotype by sex and treatment

Genotype	Sex	Noise	Level
		Low	High
C57	M	19	10
	F	22	10
F ₁	M	22	10
	F	26	10
F ₂	M	22	10
	F	22	11
JK	M	21	10
	F	21	10

Apparatus

The open field arena was a 15 x 15 cm square with 20 cm high white walls, brown paper floor, and hardware cloth cover. Illumination was provided by two 60-watt incandescent bulbs in 16.5 cm diameter reflectors. The lights were positioned one on each side of the open field, 50 cm above the arena floor and 5 cm outside the side walls.

For the emergence test, a hole 7.6 x 8 cm was made in the back wall of the home cage. An insert of material similar to that from which the cage was constructed provided a solid backwall for the cage except during testing. A cage cover of .6 cm fiberboard was used during testing. The area into which the subjects could emerge was similar to the open field arena except that a 5 cm wide guillotine door was built into one side wall. The door could be opened 8 cm and activated an electric timer that ran as long as the door remained open. Lighting was provided by fluorescent room lights. An inclined mirror mounted about 1 m above the apparatus provided a view of the inside of the arena from the experimenter's location 2 m distant.

Procedure

Testing. Each subject was tested in the open field for 5 min a day at the same time each day for 3 consecutive days. For the high stimulation group the noise was turned from zero to full-on immediately after placing a subject in the test apparatus. The volume was set back to zero at the end of the 5 min test before the subject was removed from the apparatus. Data recorded included the number of boluses in the arena at the end of the test. For emergency testing the metal insert was removed from the back of the cage and the cage was positioned flush with the guillotine door of the arena. The fiberboard cover was then placed over the cage. At least 30 sec elapsed from positioning of the cage until the guillotine door was opened to begin a test trial. A trial lasted until the subject met the emergence criterion of having all four feet on the floor of the arena or until the door had been open for 30 min. For the high environmental stimulation group the volume of the tape machine was turned from zero to full-on 10 to 15 sec before the emergence door was opened, and back to zero after the emergence crit-

erion was met. Each subject was tested at about the same time each day for three consecutive days. For both tests the apparatus was cleaned and paper floor renewed between each subject.

Data analysis. The scores used in analyses were individual subject's scores summed over the three test trials. These were total number of boluses excreted during the open field test and latency to emerge, in seconds, from the emergence test. For the defecation data, a test of heterogeneity of variances ($F_{max} = 8.89$) was not significant at the .01 level and therefore raw scores were used in analysis. A logarithmic transformation is usually recommended in order to normalize latency scores. A transformation of the form $[X = \log(x+1)]$ was applied to the emergence scores and greatly reduced the heterogeneity of variances present in the raw data. Three-way unweighted means analyses of variance (Winer, 1962) were applied to these data.

RESULTS

With regard to open-field defecation scores, highly significant main effects due to genotype ($F = 42.77$, $df = 3/240$, $p < .001$), sex ($F = 11.41$, $df = 1/240$, $p < .001$), and environmental stimulation ($F = 24.80$, $df = 1/240$, $p < .001$) were found. None of the interactions reached an appreciable significance level. Within each genotype, males defecated more than the females. These data, pooled across sex, are graphically represented in Figure 1. Inspection of Figure 1 indicates that members of the JK strain defecated much more than those of the C57 and that the hybrid generations were intermediate. In addition, the environmental stress resulted in a relatively consistent increase in defecation. These results are in agreement with most of the relevant literature concerning defecation in an open field situation. The sex difference has been reported previously (Tellegen, 1963; DeFries and Hegmann, In press). The highly significant effect of the environ-

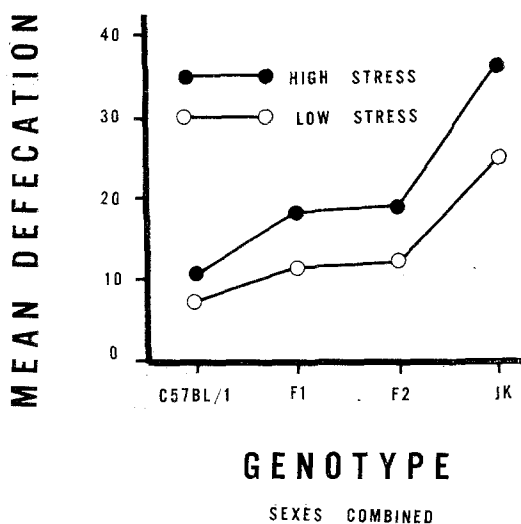


FIGURE 1. Effect of environmentally induced stress during testing on open-field defecation of each of two inbred strains of mice and their derived F_1 and F_2 generations. Defecation values are combined across sex within genotypes.

mental treatment establishes its validity in that the effect was in the direction predicted from the theoretical considerations upon which the open-field test is based (Hall, 1934; Broadhurst, 1960). A biometrical genetic analysis of these data is presented elsewhere (Whitney, 1967).

Analysis of home cage emergence latency revealed highly significant main effects of noise during testing ($F = 24.33$, $df = 1/240$, $p < .0001$) and genotype ($F = 24.81$, $df = 3/240$, $p < .0001$). The main effect of sex and all possible interactions did not approach significance. As may be seen in Figure 2, the highly

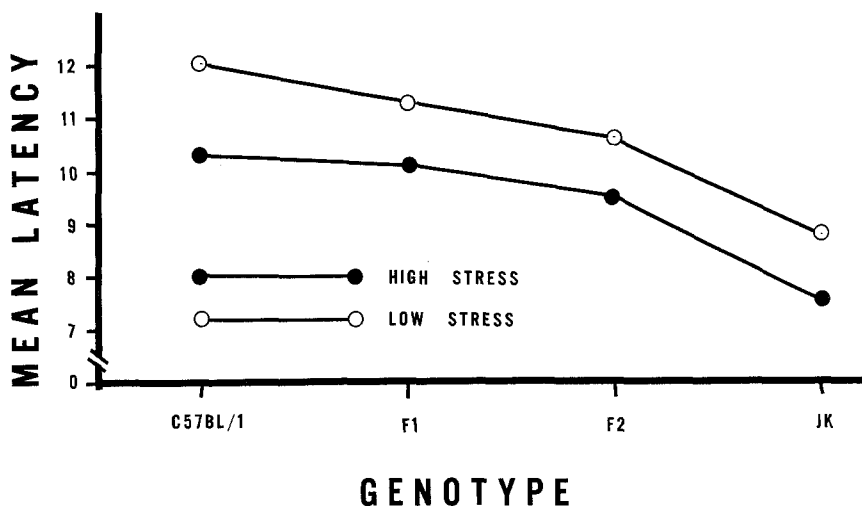


FIGURE 2. Influence of environmentally induced stress during testing on latency of home cage emergence of each of two inbred strains of mice and their derived F_1 and F_2 generations. Latency values are log. scores combined across sex within genotypes.

significant strain difference was due to the long latency to emerge of the C57 strain as compared to the fast emerging JK; and F_1 and F_2 generations were both within the range of the parental inbred strains. Increased environmental stimulation during testing affected all genotypes uniformly, leading to a decrease in emergence latency.

DISCUSSION

Previous research using the identical sublimes employed in this study has established that the C57 strain is less fearful and more active in an open field test than is the JK (Lindzey, Lykken, & Winston, 1960; Lindzey, Winston & Manosevitz, 1963). The present results demonstrate that the JK is more fearful than the C57 when tested in an open-field arena, virtually identical to the emergence arena, and that environmental stimulation is sufficient to increase fearfulness in the open-field test. However, of the strains tested by Tellegen (1963), the JK was the least timid in his emergence situation. Manosevitz (1965) also reported that the JK has a lower emergence latency than the subline of the C57 used in this study. These results are all consistent in demonstrating that the JK strain is more fearful (open-field defecation), but less timid (home cage emergence), than the C57, and that increased environmental stimulation leads to "more-JK-like" behavior.

In order to account for the lack of relationship, or indeed negative relationship, between the constructs of fearfulness and timidity, one could argue that we are dealing with two traits that are independent in the population investigated. Or, in order to maintain a conceptual relationship, one could invoke a concept of general arousal and suggest that given a certain base level of emotionality, either environmental stimuli or genetic modification sufficient to increase the level of fearfulness cause a reduction in timidity; in short, scared but rash responding. Yet another way of handling these data could be to retreat into operational definitions and consider open field defecation instead of fearfulness and home cage emergence instead of timidity. While the latter is definitely a legitimate approach, especially at early stages of investigation, it removes the investigation from the area of primary original interest; the comparative study of variables relevant to human emotionality in infrahuman species.

It is suggested here that the conceptual approach represented above, while widespread in current psychological literature, consists of a progression from *a priori* analogic reasoning to the invention of relatively arbitrary operational definitions, then through rigorous experimentation to post hoc explanations, and that the process suffers from a lack of application of a fundamental consideration of comparative research. Hodos and Campbell (1969) have recently pointed out that much of comparative psychology suffers from a failure to distinguish between members of a common evolutionary lineage and members of arbitrarily constructed phylogenetic scales. While their arguments are relevant in any consideration of the comparative approach, the present discussion is concerned with the conceptualization of variables to be investigated, rather than with the evolutionary relationship between the species utilized.

Most current research concerned with variables of animal temperament involves, either explicitly or implicitly, a comparison of the behavior of nonhuman species with the behavior of humans. Klopfer (1968) has discussed the dangers of analogic reasoning in interspecific conceptualization of behavioral variables, and Scott (1955) cogently suggested a historical explanation of the problem within the field of comparative psychology. Basically, Scott (1955) pointed out that in the development of psychology there has never been a phase of observation and description of behavior in nonhuman species. Rather, psychology developed somewhat abruptly from philosophical theorizing into rigorous experimentation. As a result, psychologists often engage in *a priori* reasoning, based on observation of human behavior, and proceed directly into methodologically sophisticated experimentation utilizing infrahuman species, but with almost total neglect to the serious consideration of the meaning of their conceptual entities in the behavioral organization of the species studied. The result is too often an experiment that requires post hoc hypothesizing and has an unknown relevance to the trait domain of original interest.

An absurd example of such an approach would be the investigation of interspecific predator behavior as a model of intraspecific (human) aggression. An example provided by Scott (1955) involves investigating operationally defined hoarding (model for human miserliness or n Retention?) utilizing species that

may not engage in behavior fitting any reasonable definition of hoarding. We can now add the example of investigating operationally defined timidity in the mouse in the almost total absence of information concerning behavioral proclivities of mice. Even in regard to the relatively well validated construct of fearfulness in rodents, an increased precision of conceptualization is possible when behavior is considered in the context of the evolutionary adaptation of the species employed (Bruell, *In press*; Scott, 1967; and Whitney, 1967).

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