trols simply remained in their cages until the training sessions. All chicks were given training sessions for imprinting at 12 and 16 hours of age. Each animal was transported to the apparatus in a container and then placed in the restraining unit for a 1-minute habituation period. The restraining unit was then removed, and the imprinting object was introduced and moved around the apparatus at a rate adapted to the following of each individual chick. At the end of 10 minutes, the chick was returned to its cage. A record was made of the strength of following, defined as the number of seconds during which the animal was in motion and within 30 cm of the object. Two 3-minute following tests were given to each chick at 30 hours and again at 54 hours of age. At each age, half the chicks were tested with the imprinting object first and the unfamiliar object second and the remaining half were tested in the reverse order. The surrogates were moved at a constant speed of approximately 30 cm per second. Each such test was preceded by a 1-minute habituation period. A record was again made of strength of following.

Our results indicate that arousal (handling) produced some increase in the amount of following during training, particularly in group H5. However, differences were not statistically significant. During the later test sessions, handling at 5 hours of age produced a clear increment in following, while handling at 9 hours produced no observable effect.

Median following scores in seconds for each of the four groups are shown in Fig. 1. Since we found no significant differences in the strength of following of the imprinting object as opposed to the nonimprinting object, we have combined these scores. In addition, since differences between groups were almost identical for the tests at 30 and at 54 hours of age, scores for these two sessions have also been combined. Statistical analysis of the resulting data in Fig. 1 showed that the animals in group H5 followed significantly more than those in group C5 ($U = 34.5$, $p < .05$). However, there was no difference between chicks handled at 9 hours (group H9) and the 9-hour controls (C9). Since this result applies equally to both objects, it indicates that the effects of arousal prior to training were apparently nonspecific.

The data reported here give definite, though limited, support to the hypothesis of early arousal. It is possible that the comparatively slight effects of arousal on following during training were due to our method of training, that is, adapting the speed of the surrogate to each chick. Alternatively, such differences as were evident during training may have been real in the sense that they were an initial reflection of the significant effects found in the later tests.

While the results of our experiments hold for mild tactile stimulation administered at specified ages, it remains to be seen whether arousal involving other modalities and of different intensity will have the same effects.

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Inheritance of Avoidance Conditioning in Mice: A Diallel Study

Abstract. *Significant genetic differences were demonstrated in the rate of avoidance conditioning among offspring from all 25 mating combinations of 5 highly inbred mouse strains. Hybrids of C3H parents learned fastest, while those of A/JAX parents learned slowest. Most hybrids learned better than either parent. Evidence that differential influences of the early maternal environment affected the rate of learning was not supported.*

Although most genetic research has been concerned with the study of morphological and physiological characteristics, there is considerable current interest in the inheritance of behavior characteristics. Behavioral differences have been demonstrated among highly inbred strains of mice and their respective F_1 crosses in areas of locomotor activity, exploration of novel environments, and other relatively simple classes of behavior (*1*). Whereas some recent evidence implicates the importance of hereditary differences in learning behavior (*2*), most previous studies considered only a few pure strains, or two pure strains and their crosses. Consequently, the findings may be applicable only to the strains chosen and may not be representative of genetic relationships in differing strains. The objective of this investigation was to study the range of genetic influence on learning in mice by using a diallel mating system in which all intercrosses among a set of inbred strains were tested for the acquisition of a learned avoidance habit.

Ten males and 10 females from each of the 25 mating combinations of the following 5 pure strains of mice were tested for acquisition of avoidance conditioning: A/JAX, BALB/c, C3H, C57BL/10, and DBA/1. A total of 500 mice were tested. At approximately 100 days of age, each subject was placed in a shuttle-box, measuring 26.5 by 5 by 4 cm, and received 200 consecutive avoidance-conditioning trials. The conditioning stimuli were a muffled buzzer and a 3000 cy/sec sine-wave tone which began 2.5 seconds after the onset of the buzzer. Electric shock was programmed to be delivered to the grid floors 5 seconds after the onset of the first cue. A jumping response across a continuously charged pit during the first 5 seconds of each trial activated a photosensitive control system which postponed shock for that trial and was considered an avoidance response. A pit-crossing after 5 seconds terminated the cues and shock and was considered an escape response. The interval between trials was fixed at 30 seconds. Four mice were trained simultaneously according to this schedule in separate automatic testing chambers. The score for each subject was the number of avoidances in 200 trials.

The data were arranged in a 5 by 5 square design, in which the rows represented the genotype of sires, and the columns, the genotype of dams. The 25 squares thus formed represented the 25 genotypic combinations from all matings of the 5 pure strains, and included 20 F_1 crosses and the 5 pure strains. Two complementary statistical models were used to assess the data. Model 1 represented a factorial analysis of variance by which the genetic effects (gametes) of dam and sire could be evaluated independently of each other. Model 2 represented an extension of Griffing's combining abilities analysis of variance, which enabled both sexes of offspring and resulting interactions to be included (*3*). By this model the effect of the five pure strain gametes upon learning was assessed simultaneously in sires and dams. Table 1 presents a summary of the statistical tests.

Wide differences in learning ability were evident among the 25 genotypes. Figure 1 illustrates the avoidance conditioning scores for each of the 20 hybrid crosses in comparison with the scores of the respective parental strains. The avoidance means of the highest scoring genotypes, C3H × DBA/1, DBA/1 × C3H, and C57BL/10 × C3H, were approximately eight times the mean of the lowest scoring pure strain, BALB/c. The highly significant dam and sire effects, as well as the significant general combining abilities effect in Table 1, demonstrate that the strain of the parents considerably influenced the level of performance of the F_1 crosses. The performance score of hybrids in which there was a C3H parent was approximately 12 percent higher than the mean of all crosses. Conversely, hybrids in which there was an A/JAX parent performed 7 percent below the mean of all crosses.

The highly significant dams × sires interaction and the specific combining abilities effect indicate that the scores of certain genotypes were statistically different from an expectation based on the observed additive effects of the parental genes. This interaction was primarily due to the low scores of the five pure strains and the unusually high performance of C3H × DBA/1 hybrids.

Female mice were superior to males; females averaged 26.9 percent avoidances while males averaged 19.3 percent. The absence of any significant genetic interactions with sex of offspring indicates that essentially identical conclusions could have been drawn from studying either sex of offspring alone. In particular, the absence of the reciprocal × sex interaction indicates that sex-linkage did not affect avoidance acquisition. However, other findings at this laboratory indicate that sex-linked genes in C3H × DBA/1 hybrids do have some effect on avoidance extinction as well as on the weight of the adrenal gland (*4*).

To test the effect of possible influences of the early maternal environment upon learning uncomplicated by genetic variables, orthogonal contrasts were formed between individual pure strain dam and sire total scores (row and column totals). This test indicated that no significant differential maternal effects were associated with rearing differences among the five pure strain mothers ($p > 0.10$; $F = 1.94$, $df = 4{:}350$). However, cross-fostering techniques would be needed to evaluate maternal influences in greater detail.

An indication of the mode of inheritance underlying a quantitative characteristic may be obtained by comparing the mean for each hybrid against the average of the respective parental scores (midparent average). If the hybrid score is the same as the midparent value, or lies between it and the higher scoring parent, the mode of inheritance is termed intermediate or partially dominant, respectively. If the hybrid score is equal to, or exceeds the score of the higher scoring parent, the mode of inheritance is called dominant or over-dominant, respectively. In 15 of the 20 comparisons in Fig. 1, the learning performance of F_1 crosses was higher than the midparent average, and in 14 cases, the hybrids exceeded the score of the higher-scoring parent. A statistical evaluation of the 20 comparisons by orthogonal contrasts demonstrated that F_1 crosses learned to avoid shock to a higher degree than would be expected on the basis of the predicted additive effects of the parental genes ($p < 0.0001$; $F = 51.9$, $df = 1{:}450$). Thus, the overall mode of inheritance of avoidance conditioning in F_1 crosses was dominant, and in most cases was over-dominant.

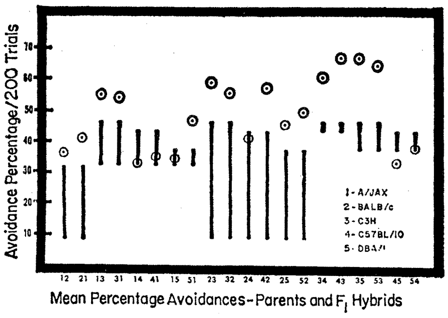

Fig. 1. Average scores for individual hybrid groups compared with the mean scores of the respective pure-strain parents. The circled dot represents the score of ten male and ten female hybrid mice; the endpoints of each line represent the learning scores of the respective parental strains. The first number of each F_1 designation indicates the strain of dam; the second number refers to the strain of sire. For example, 12 is the F_1 cross from the mating of an A/JAX female to a BALB/c male.

The superiority of the F_1 cross compared to the midparent expectation, usually called heterosis, or hybrid vigor, has been repeatedly demonstrated in hybrid progeny obtained from the crossing of highly inbred lines. Whether this heterosis arises from interlocular interactions, the complementary gene hypothesis, or from intralocular interactions, the hypothesis of overdominance, cannot be settled from these data (*5*). Since quantitative behavioral differences most probably arise from polygenic origin rather than from single-gene action, either hypothesis or both may account for the observed heterotic effect. Whatever the mechanism or genetic route, the important finding is that heterozygotes are clearly superior to their inbred parents in avoidance learning. Aside from the practical consideration of breeding faster-learning mice, the observed hybrid vigor suggests that avoidance learning may be of significant adaptive value to a species. Bruell has theorized that only quantitative polygenic traits, which were biologically adaptive and under past selection pressure, would result in heterotic inheritance, and that traits which possessed negligible adaptive value would be inherited in an intermediate manner (*6*). This suggests that the degree of heterosis induced by crossing inbred lines may be a useful index of the biological adaptiveness of a quantitative phenotype.

This study suggests that behavior-

Table 1. Summary of statistical evaluations of avoidance conditioning performance among 25 genotypes of mice. Analyses according to models 1 and 2.

Source	*df*	Mean square	F-ratio
	Model 1		
Dams	4	5,839	16.82*
Sires	4	5,131	14.78*
Sex	1	25,891	74.57*
Dams × sires	16	2,923	8.42*
Dams × sex	4	93	0.27
Sires × sex	4	625	1.80
Dams × sires × sex	16	401	1.16
	Model 2		
GCA†	4	10,310	29.70*
SCA‡	10	4,366	12.58*
Reciprocals	10	574	1.65
Sex	1	25,891	74.57*
GCA × sex	4	408	1.18
SCA × sex	10	437	1.26
Reciprocals × sex	10	328	0.95
Error	450	347	
Total	499		

* $P < .0001$. † General combining ability. ‡ Specific combining ability.

genetic research in conjunction with morphological and physiological studies may be of greater value in the investigation of evolutionary theory than morphological and physiological research alone.

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Pupil Size in Relation to Mental Activity during Simple Problem-Solving

Abstract. *Changes in pupil size during the solving of simple multiplication problems can be used as a direct measure of mental activity. The pupil response not only indicates mental activity in itself but shows that mental activity is closely correlated with problem difficulty, and that the size of the pupil increases with the difficulty of the problem. These findings relate to recent Russian research on the pupillary reflex in connection with orienting and brain stimulation.*

We reported previously that while a subject is viewing visual material pupil dilatation serves as an excellent indicator of the interest value of the material to the subject (*1*). In that study, we demonstrated both a differentiation of stimuli (more or less interesting) and a differential interest value of the same stimuli for subjects of different sexes. It was pointed out that this phenomenon is completely independent of pupillary activity as regulated by the external light conditions, under the control of the parasympathetic nervous system, but is instead governed by the sympathetic nervous system, as are other emotional responses. Since Shachnowich (*2*) reports pupillary constriction as being part of the orienting reflex to novel visual stimuli, we thought that the dilatation found in the previous situation could not be attributed to any novelty factor of the stimuli, but must be a measure of a more basic reaction on the part of the subject. Sokolow (*3*) emphasizes the close relationship between the orienting reflex and unconditioned adaptive reflexes, clearly differentiating the two responses.

In reporting on their extensive work on the pupillary reflex, Lowenstein and Loewenfeld (*4*) concluded that the "pupillary reflex does not depend upon the activity of an isolated reflex arc, unaffected by central nervous events, but is a complex function." They also report that one of the factors which leads to deviations from a pupil size which would be predicted on the basis of the amount of light striking the eye is emotional excitation in the subject.

One of the earlier papers indicating that pupil size might be an indicator of the degree of activity of the central nervous system was that of Bumke (*5*) who discussed the work of Roubinowitsch, which was similar to earlier observations made by Exner. They observed the eyes of subjects who were engaged in mental activity, and saw gross changes in pupil size which seemed to be correlated with this activity.

In the experiment reported here, simple mathematical problems were used as the material for mental activity. By utilizing photographic techniques, we were able to determine exact changes in pupil size as well as the exact moment at which changes occurred.

Four men and one woman served as subjects in the experiment. Presumably all were above average in intelligence; one held a Ph.D. degree, two were at an advanced graduate level, one held a B.A. degree, and one was an undergraduate research assistant in the psychology department of this university.

A standard metal head-holder, cushioned with foam rubber, was attached to a laboratory table. Fifteen centimeters from the head-holder was a front-surface mirror on a ring-stand clip. Both head-holder and mirror could be adjusted to the height of the subject. The mirror was always below the subject's line of vision. It was tilted 10 deg from the vertical plane and was at a 45-deg angle in relation to the center of the subject's face and just below the right eye. The mirror reflected the subject's eye into the camera lens 39 cm away. The camera was a 16-mm Arriflex with a 150-mm Kilfitt *f*/3.5 lens in an adapter. The camera was run by an animation motor and took two frames per second with a ¼-second exposure.

The eye was illuminated by a standard 100-watt bulb in a holder with a dulled reflector taken from a gooseneck lamp. Light was directed into the subject's face, with the holder resting on the tabletop and the bulb 45 cm from the subject's eye. Except for this light the room was in darkness. The amount of light falling on the eye was 990 lumen/m^2, making it possible to use an exposure of *f*/8 with Eastman Royal Pan film (800 ASA).

A Pola-Coat screen was 1.45 m from the head-holder. Projected onto the screen from the rear was a 35-mm slide, dark gray, with a white number "5" in the center. The image was horizontal and 16 × 25 cm in size. A Sawyer projector with a 12-cm lens was placed 100 cm from the screen. The number in the center of the slide was 47 cm from the top of the table and clearly visible to the subject.

The subject entered the experimental room and was seated at the table. Any necessary adjustments were made in the height of the mirror or head-holder. The subject was then told that he would have his head in the holder for 3 or 4 minutes, and was requested to remain as motionless as possible, fixating on the number "5" on the screen in front of him. He was told that he would be given a mathematical problem to solve, and that he was to give his answer orally when he had reached a solution.

While one experimenter operated the camera, a second gave the instructions and the problems. The subject was also told whether or not his answer was correct. A third experimenter recorded the number of the frame at which each question was given and answered from the frame counter on the animation motor. After starting the camera, nothing was said for 30 seconds. The first problem was then given. After each answer, 5 to 10 seconds elapsed before the next problem was given. The problem was always given when the counter was at an even 20-frames interval. Since the time taken for solving the problem